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# POLYPLOIDY, LIFE-FORM AND VEGETATIVE REPRODUCTION

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IN the concluding chapter of my monograph on »Apomixis in higher plants» (Lund, 1948) I have ventured to re-interpret some fundamental aspects of natural polyploidy and to draw up in rough outlines a phylogenetical picture of the phanerogam species in their various aspects of transformation. In this paper further information will be presented, supporting and advancing the conclusions of the publication mentioned.

## I. POLYPLOIDY IN WEED FLORAS.

Following KORSMO (1926, 1930), I will here define such plant species as weedy which intrude upon the cultivated plants, damage them and spoil their yield production more or less. It is necessary, says KORSMO (l. c., p. 1), to extend the type of soil making the species a weed somewhat further than to cultivated ground, since many species, growing along railroads and highways, in house-yards, wharfs, etc., rather easily spread into cultivated areas and then become noxious intruders.

Biologically viewed, the plant species denoted as weeds are characterized by very successful methods of dispersal, whether this is effected by seeds alone or by seeds and propagules in combination. Weeds easily adapt themselves to widely variable climatic conditions. Often they are highly resistant to exterior influences, or the preparing of the soil, leading to severe damages of the vegetative system, may even help to rejuvenate and propagate them. Many increase their areas of distribution rather abruptly and are then able to persist for long periods in their new localities.

KORSMO divides the phanerogamous weeds into three groups: (1) Annual and biennial species, exclusively reproducing by seed, (2) perennial species more or less incapable of vegetative dispersal (»stationary weeds»), and (3) perennial species possessing an uninterrupted capacity of vegetative spreading (»root-wandering weeds»). In the third group we find species which often turn into real pests, species

like *Agropyron repens*, *Phragmites communis*, *Urtica dioica*, *Cirsium arvense*, *Sonchus arvensis*, *Tussilago Farfara*. It must not be forgotten, however, that several of the species belonging to KORSMO's second group propagate by adventitious buds formed from damaged roots or reproduce by agamospermy.

The following survey gives the chromosome conditions of the different groups:

| | Seed-reproducing annuals and biennials | Stationary perennials | Root-wandering perennials | Average |
|---|---|---|---|---|
| Rate of polyploidy | 34 % | 46 % | 64 % | 44 % |
| No. of species .... | 83 | 35 | 39 | 157 |

(Only those species which exclusively occur as polyploid are included in the percentages above.)

Similar results were obtained in an analysis of the Swiss weed flora (GUSTAFSSON, l. c., p. 295). The Swiss weeds of annual or biennial structure consist to 32 % of polyploids. The more or less stationary perennials show 38 % and the wandering perennials 64 % polyploids.

The weed flora of Canada (CLARK and FLETCHER, 1909) behaves in a similar manner:

| | Seed-reproducing annuals and biennials | Stationary perennials | Root-wandering perennials | Average |
|---|---|---|---|---|
| Rate of polyploidy | 32 % | 38 % | 68 % | 40 % |
| No. of species .... | 85 | 29 | 25 | 139 |

There is consequently no doubt that perennial weeds with a pronounced capacity of vegetative propagation consist of polyploids to a very high extent — in fact, about twice as frequently as the annual and biennial species. Perennials lacking stolons and runners occupy a middle position. This result is in line with the conclusions drawn in my monograph on apomixis. According to my mode of interpretation, the clonal reproduction facilitates the origin of polyploidy. Polyploidy in its turn augments an already existing vegetative spreading.

In my discussion on polyploidy in closely defined areas (GUSTAFSSON, l. c., p. 276) it was pointed out that many species exhibiting a pronounced mode of vegetative propagation, *although diploid*, often possess a higher basic number than such species as rely on seeds for their dispersal. This result holds true for the weedy species, too.

Sixteen per cent of the annual and biennial weeds of Scandinavia have a basic number of 11 or more, against 22 % in perennial weeds without stolons and runners, and no less than 50 % in perennials which migrate rapidly by vegetative means. Evidently the vegetative methods of reproduction are increased in intensity, both by processes of polyploidy and processes raising the basic number. Annual and biennial species generally keep to the diploid state or they even actively decrease their basic number, as concluded for species in *Crepis*.

KORSMO enumerates fourteen weedy grass species. Six are annual or biennial; two of them exclusively occur as polyploid (33 %). Eight grass species are perennial; here the polyploids amount to six (75 %).

## II. POLYPLOIDY IN HEMEROPHILOUS AND HEMEROPHOBOUS SPECIES.

In his publication of 1916 dealing with the influence of culture on the phanerogam flora LINKOLA studied the different plant elements in a region north-east of the Ladoga Lake and compared the conditions of this province with those prevailing inland. The Ladoga area shows a fertile soil of conspicuous culture character and harbours 613 species. The inland area contains an original, rather poor flora with 497 species.

LINKOLA divided the phanerogamous species of these two regions into the following groups:

(I) Species which profit from culture with regard to their occurrence and spread — *hemerophilous species.*
  (a) Introduced species — *anthropochorous species.*
  (b) Species occurring as original but easily turning hemerophilous — *apophytic species.*

(II) Original species neither favoured nor disfavoured by man's influence — *hemeradiaphorous species.*

(III) Species distinctly suffering from culture — *hemerophobous species.*

In my analysis of LINKOLA's data I have not entirely followed his method of computation. In this paper a species is ranked as anthropochorous or, as the case may be, apophytic when it occurs as such in either or both of the two regions. (Cryptogams and gymnosperms, as well as microspecies of *Hieracium* and questionable cases, have been excluded from the table.) The Carices are treated separately.

| | Rate of polyploidy in | | | | |
|---|---|---|---|---|---|
| | Graminaceae | Other monocotyledons | Dicotyledons | Average | No. of species |
| Anthropochorous species | 72 % | 75 % | 38 % | 41 % | 180 |
| Apophytic » | 63 | 45 | 47 | 49 | 172 |
| Hemeradiaphorous » | 88 | 56 | 49 | 54 | 94 |
| *Hemerophobous* » | *57* | *36* | *29* | *33* | *66* |

The result of this investigation goes to show that the hemerophobous phanerogams contain fewer polyploids than the hemerophilous species. This is true of all kinds of species: grasses as well as other monocotyledons and dicotyledons. The hemeradiaphorous species, equally adapted to original and cultivated conditions, possess the highest average rate of polyploidy.

The genus *Carex* consists of one anthropochorous, thirteen apophytic, eleven hemeradiaphorous and seven hemerophobous species with their somatic numbers determined. The average chromosome numbers are 66, 61, 62 and 59 respectively, i. e. the hemerophobous species occupy a less derived position.

Man is an active agent in transforming the natural floras. Polyploids profit more or less from his influence.

## III. POLYPLOIDY IN SNOW-LAYER FLORAS.

The idea that extreme climates induce a rich formation of polyploids was advanced by HAGERUP in 1932 and then further elaborated by TISCHLER in his computations. GUSTAFSSON (l. c., pp. 280—286) opposes this view and holds that polyploids accumulate in certain regions, not owing directly to the extreme climate *per se* but rather to a break-down of the diploid sexual mechanism and an exaggeration of the vegetative system. An analysis of the Scandinavian mountain plants shows that the very common species largely keep to the diploid state. In fact, they display a lower rate of polyploidy than average lowland plants.

In order to elucidate this question further, the snow-layer flora of Knutshö (RESVOLL, 1917) was analysed with regard to its polyploid conditions. In her studies on alpine and arctic plant species, she examined the flowering and seed-setting of Norwegian mountain species growing in about twenty snow-layers of Knutshö in the Dovre massif, all of them situated 1.400 to 1.550 metres above sea-level. Here the melting of the snow does not begin until late in summer, so that the

plants have an extremely short period of growth at their disposal. No doubt, cold and frost often interfere with meiosis, sex-cell formation and fertilization. Unreduced gametes, with polyploid embryos as a consequence, ought to arise rather frequently. None the less, we find that 20 out of 41 sexual species (Carices, agamosperms and viviparous species excluded) are diploid, i. e. the rate of polyploidy is 51 %, lower than for the average Scandinavian mountain species and not appreciably higher than in monocotylous and dicotylous lowland plants. Consequently, the extreme conditions do not markedly favour polyploidy.

We also find that in the snow-layers of Knutshö diploids are wider spread than high-polyploid sexuals. They occur as abundantly as the agamospermous and viviparous complexes, which to a less extent than sexual species depend on long and warm summers for their seed-setting.

| | 2x-sexuals | 4x + 6x-sexuals | ≧8x-sexuals | Apomicts |
|---|---|---|---|---|
| No. of species ..... | 20 | 15 | 6 | 8 |
| Average occurrence: | | | | |
| No. of snow-layers .. | 5,2 | 3,3 | 2,0 | 5,1 |

Consequently, there is no reason whatever to suppose that polyploids are favoured by the harsh conditions prevailing in this extreme type of environment.

The data gathered by NORMAN (1895—1901) and cited by RESVOLL allow of a similar conclusion. He listed the species occurring in snow-layers of arctic Norway north of the Polar Circle.

*Very common* were 6 diploid and 1 polyploid sexuals, together with 2 agamospermous and bulbiliferous complexes.

*Common* were 4 diploid and 3 polyploid sexuals, together with 2 agamospermous and bulbiliferous complexes.

*Not rare* were 5 to 7 diploid and 5 polyploid sexuals.

*Rare* were 5 diploid and 6 polyploid sexuals.

## IV. POLYPLOIDY IN BEACH FLORAS.

In his publication of 1937 ROHWEDER considered that he was justified in concluding that polyploids are superior to diploids in their adaptive capacity to difficult life-conditions (»schwierige Lebensräume»). He examined the flora constituents of the Darss-Zingst area in the German province of Vor-Pommern. In the West and North it is washed by the open Baltic Sea and separated from the mainland by

so-called »Bodden» (arms of the Sea peculiar to this part of Germany). A re-analysis allows quite deviating conclusions.

The typical beach flora (ROHWEDER, l. c., Table 1) shows a higher degree of polyploidy than the dry regions of Altdarss and the Darsser moor (Table 2), the moss and fir flora of Neudarss (Table 3) and the dune flora of Darss-Zingst (Table 4). The beach flora contains 65 to 70 % of polyploid species, in contrast to the other regions, which do not show more than 40 to 50 %. The flora constituents along the road crossing the Darsser Riff give a corresponding figure of 55 to 60 %. (All Carices and agamospermous complexes are omitted.) The polyploids are said to occupy no less than 92 % of the area along the beach zone. This fact was taken to indicate a higher »vitality» of the polyploids.

In my monograph on apomixis it was pointed out that hydro- and helo-phytes possess a considerable capacity of vegetative reproduction and parallel to this faculty have become polyploid, or at least have acquired high basic numbers. Polyploidy favours the vegetative system. This in its turn favours polyploidy. As a consequence the very common and competitive water plants attain a polyploid status. But the polyploid abundance cannot be regarded as evidence of a higher »vitality» *per se*, since the seed-setting is often very poor. Such is, for instance, the case in the common reed, *Phragmites communis*, which spreads almost entirely by vegetative means. Appropriate definitions of »vitality», »natural fitness», etc., must include the generative as well as the vegetative system.

Fourteen beach species are hydro- or helo-phytic (applying RAUNKIAER's terminology). Twelve (86 %) are polyploid. The remaining two species have high basic numbers ($x = 11$, *Lycopus europaeus*; $x = 15$, *Typha angustifolia*).

ROHWEDER's data indicate a close correspondence of high »vitality» and polyploidy:

| Degree of vitality | 3–5 | 2 | 1 |
|---|---|---|---|
| Rate of polyploidy .... | 88 % | 72 % | 54 % |
| No. of species ..... | 16 | 39 | 48 |

The following species belong to the group of 3—5 point »vitality»:

*Typha angustifolia*
*Potamogeton filiformis*
*Triglochin maritimum*
» *palustris*
*Phragmites communis*
*Alopecurus geniculatus*
*Agrostis stolonifera*
*Scirpus maritimus*

| | |
|---|---|
| *Scirpus Tabernaemontani* | *Calystegia sepium* |
| *Juncus glaucus* | *Mentha aquatica* |
| » *lampocarpus* | *Achillea millefolium* |
| *Potentilla anserina* | *Bidens tripartitus* |

The two diploids included are *Typha angustifolia* and *Calystegia sepium*. Both possess high basic numbers and, in addition, show a pronounced capacity of vegetative propagation. The therophytic *Bidens tripartitus* is the single species that lacks the vegetative mode of dispersal. *Alopecurus geniculatus* is a biennial or perennial hemicryptophyte, which may propagate by vegetative means. All other species possess an intense regenerating and spreading capacity. Corresponding to this faculty, they show high chromosome numbers (4x, 6x, or more).

Consequently, the high »vitality» of polyploid beach plants depends on an exaggeration of the vegetative system, favouring the local spreading by asexual methods of reproduction.

Exactly similar results are obtained by a close analysis of the data gathered by ENGLUND (1942) on the shore flora of Gotland, the large island in the middle of the Baltic Sea.

The author mentioned grouped the phanerogam species according to their demands for moist soil. The figure *4* denotes hydrophytic, *3* denotes hygrophytic, *2* mesophytic and *1* xerophytic conditions. We find the following rates of polyploidy:

| Degree of moisture | 3—4 | 2 | 1 | Average |
|---|---|---|---|---|
| Rate of polyploidy ..... | 71 % | 48 % | 50 % | 55 % |
| No. of species (apomicts and Carices excluded) ..... | 35 | 60 | 46 | 141 |

Consequently the hydro- and hygro-phytic constituents of the shore flora are polyploid to a high extent.

ENGLUND has given every plant species a figure from 1 to 10 according to its general abundance. Calculating the rate of polyploidy and the number of annual and biennial species having the diploid number, we find the following conditions:

| Abundance | 10+9 | 8+7 | 6+5 | 4+3 | 2+1 |
|---|---|---|---|---|---|
| No. of species ..... | 15 | 24 | 36 | 40 | 22 = 137 |
| Rate of polyploidy ..... | 67 % | 71 % | 56 % | 53 % | 36 % |
| No. of annual and biennial species having the diploid number ..... | 0 % | 17 % | 17 % | 15 % | 27 % |

It is evident from this table that a shore plant, in order to become common and abundant, should be polyploid. This is not owing to any superiority of the polyploid status, but owing to the capacity of vegetative reproduction that is manifest in many polyploids. The commonest diploids, too, reproduce rapidly by stolons, runners, creeping rhizomes, or other kinds of propagules. Such is the case with *Sagina nodosa* (?, x = 11), *Trifolium fragiferum* (x = 8), *Glaux maritima* (x = 15), *Galium palustre* (x = 12), *Cirsium arvense* (x = 17). (Note that four of them have high basic numbers.) Owing to the exaggerating effect of polyploidy upon the vegetative system, the vegetative reproduction becomes more effective in high-polyploid species like *Triglochin maritimum* (8x), *Festuca rubra* (6x), *Agropyron repens* (6x), several *Scirpus* and *Juncus* species, *Rumex crispus* (6x), *Sonchus arvensis* (6x), *Tussilago Farfara* (6x) and, in addition, in numerous tetraploids (for instance, *Agrostis stolonifera*, *Urtica dioica*, *Potentilla anserina* and *reptans*, *Trifolium repens*, *Vicia cracca*, *Prunella vulgaris*).

The outstanding result of this discussion is that beach floras, and especially their hydro- and hygro-philous elements, are preferably built up on polyploids. This has no connection whatever with any extreme changes in environment, either frost or heat. Polyploids are, in fact, represented more frequently in beach than in mountain and snow-layer floras.

## V. THE EVOLUTIONARY TRENDS OF SOME SCANDINAVIAN PHANEROGAM GENERA.

To test the connection between life-form, vegetative reproduction and polyploidy further I have scrutinized the conditions in thirty-eight Scandinavian plant genera showing a wide range in chromosome number and simultaneously possessing different methods of reproduction: sexual seed formation in annual, biennial and perennial status, vegetative reproduction (including bulbilifery) and agamospermy. These genera are treated below according to their position in the system.

*Poa*. — *Poa annua* (2n = 28), of the isolated section Ochlopoa, appears as an annual, biennial or perennial species, in agreement with its postulated origin as an amphiploid between the annual *P. exilis* and the perennial *P. supina* (both having 2n = 14; NANNFELDT, 1937). No annual species possess a higher chromosome number than 4x.

Diploid perennials are *P. supina* (*v.* above), *Chaixii*, *remota*, *trivialis*. All are sexual. They possess a certain, more or less conspicuous vegetative reproduction.

The sexual perennials include *P. flexuosa* with 2n = ca. 78. This is a high-alpine species without any pronounced vegetative spreading capacity. All other Scandinavian polyploid perennials have developed highly effective modes of vegetative propagation: runners (especially in *P. pratensis, arctica, compressa,* but also in *P. nemoralis, glauca* and *palustris*), vivipary (in *P. pratensis, arctica, alpina, bulbosa,* and several hybrid products), or the perennials form seeds by agamospermous means (all the species mentioned except *P. bulbosa*). The polyploidy runs into high numbers, up to 2n = 100 or more.

*Bromus.* — In this genus the annuals and biennials have been able to develop a high degree of polyploidy. Many species spread weed-like over great parts of the world. Diploid species are *B. tectorum, sterilis* and *arvensis.* A diploid or polyploid species is *B. secalinus* (2x, 4x). Polyploid are *B. racemosus* (4x), *commutatus* (4x, 8x), *hordeaceus* (4x) and *lepidus* (4x).

The single diploid perennial is *B. ramosus,* without any pronounced mode of vegetative reproduction. *Bromus erectus* with 6x or 8x behaves in like manner, in contrast to *B. inermis* (6x, 8x, 10x), which propagates by runners.

The difference between the annual and perennial groups of species is not very profound (50 % against 63 % polyploids, 2x—8x against 2x—10x).

*Juncus.* — All Scandinavian species, whether annual or perennial, possess high chromosome numbers, up to 100 or more. *Juncus bufonius* with 2n = ca. 60 is a strict annual. Its variety *ranarius* with 2n = ca. 120, although generally regarded as annual, has another rhythm of development; it is capable of over-wintering and producing second-year shoots and flowers (WULFF, 1937).

*Rumex.* — High-polyploidy is common in the subgenus Lapathum.

Two winter-annual species are known, *R. palustris* and *maritimus,* both tetraploid.

The perennials consist of *R. sanguineus* and *conglomeratus* (2x), *obtusifolius* (4x), *domesticus* and *crispus* (6x), as well as *Hydrolapathum* and *aquaticus* (ca. 20x). The Scandinavian polyploids are commoner than the diploids. They possess high regenerating and spreading capacity. The highest polyploid species (2n = ca. 200) are helophytes.

The representatives of subg. Acetosa (*R. thyrsiflorus* and *acetosa*) are diploid. Both possess vegetative modes of reproduction.

Especially interesting are the conditions within subg. Acetosella, with its wide range in polyploidy (from 2x up to 8x).

The diploid species *R. angiocarpus* has a large area of distribution, but is not known from Scandinavia. It occurs in all five continents and is more widely spread than any polyploid (LÖVE, 1943). Similar to *R. acetosa*, it forms new shoots solely from the top of the main root. The tetraploid *R. tenuifolius* shows a certain though low frequency of vegetative root-shoots. In the hexaploid, *R. acetosella*, such shoots are formed in great numbers. This species belongs to the pronounced »root-wandering weeds». The octoploid *R. graminifolius*, which is arctic in distribution, forms root-shoots in an even higher frequency. In LÖVE's cultures (l. c., p. 108) such shoots already appeared in very young seedling stages.

These species afford striking evidence of the connection between polyploidy and vegetative dispersal.

*Polygonum.* — The chromosome conditions are known in eight annual species. Diploid species are *P. tomentosum, Hydropiper, dumetorum. P. Convolvulus* is diploid and tetraploid. Tetraploid species are *P. minus, persicaria* and *nodosum. Polygonum aviculare* is tetraploid and hexaploid. The last-mentioned species accompanies civilized man everywhere. Four out of eight species (50 %) are exclusively polyploid.

All Scandinavian perennials are polyploid (100 %), beginning with the tetraploid *P. Bistorta* with its curved rhizome and stunted internodes, proceeding to the hexaploid *P. amphibium* with its long creeping rhizomes, inhabiting wet soil and water bodies, and finishing with the octo- or deca-ploid *P. viviparum* that seldom sets seed but reproduces by means of bulbils formed in the inflorescence.

*Chenopodium.* — Most Scandinavian species are annuals. Diploid species are *C. suecicum, Vulvaria, polyspermum, murale, hybridum* (foreign representatives are denoted as tetraploid), *glaucum.* The common pig-weed, *C. album*, is generally diploid. Some alien forms seem to be tetraploid or hexaploid. An exclusively tetraploid species is *C. rubrum.*

The single Scandinavian perennial, *C. Bonus-Henricus*, is a tetraploid, developing numerous stems from the root-collar.

*Stellaria.* — Two annual species, *S. neglecta* and *apetala*, are diploid. A third one, the chickweed (*S. media*), is a derived tetraploid ($2n = 40$—$44$). This species has some capacity of vegetative spreading, since its branches may strike root. This happens very rarely in the diploids.

The perennials are predominantly diploid (*S. nemorum, Holostea, Alsine, graminea, longifolia, crassifolia, humifusa*) and often possess a

pronounced vegetative dispersal. The tetraploid *S. calycantha* develops numerous long thread-like runners. In Scandinavia the high-polyploid *S. crassipes* with 2n = ca. 100 resorts to vegetative methods for its dispersal, since seeds do not generally form. *Stellaria palustris*, with 2n = ca. 130, develops numerous stems from the rhizomes, which are branched and creeping.

Evidently the perennials have been able to develop an extremely high-polyploid condition. The correlation of growth habit and chromosome number is further accentuated by the basic number, which is 11 in the annuals, but 12 or 13 in the perennials.

*Cerastium.* — Winter-annual species are *C. tetrandrum* and *semidecandrum* (2n = 36), as well as *C. glomeratum* and *subtetrandrum* (2n = 72).

The perennial species consist of *C. cerastoides* (2n = ca. 40), *alpinum* (2n = 72), *arvense* (2n = 72), *fontanum* **scandicum* (2n = ca. 144) and *C. holosteoides* (2n = 144). Vegetative reproduction is well expressed in *C. arvense* with its numerous runners. In *C. holosteoides* there occurs a pronounced root-formation along the stems. In addition, the mother plant easily splits into several daughter-individuals, which obtain an independent life (PERTTULA).

*Sagina.* — Annual species are *S. ciliata*, *apetala*, *procumbens* and *maritima*. All are diploid, with 2n = either 12 or 22.

The perennial species have somatic numbers ranging from 2n = 22 (possibly 2n = 18) in *S. saginoides* and *subulata*, over 2n = 56 in *S. nodosa* (which is said to include diploid types) up to very high numbers (2n = ca. 100) in *S. caespitosa* and *intermedia*. *Sagina nodosa* reproduces intensively by vegetative means.

Note that the basic number of the perennials is x = 11, against x = 6 and 11 in the annuals.

*Ranunculus.* — Annual diploids are *R. ophioglossifolius* and *sardous*, annual tetraploids *R. arvensis* and *sceleratus*.

The perennials divide into a diploid and a polyploid group. The former contains *R. trichophyllus*, *hederaceus*, *glacialis*, *platanifolius*, *lapponicus*, *pygmaeus*, *polyanthemus*, *bulbosus*, *Cymbalaria*. Diploid and tetraploid species are *R. acris* (predominatingly 2x) and *R. repens* (predominatingly 4x). Some have a more or less pronounced capacity of vegetative reproduction. This is especially intense in *R. repens*.

As far as we know, most polyploid perennials have remained sexual. Such is the case in *R. peltatus*, *obtusiflorus*, *Pallasii*, *hyperboreus*, *lanuginosus*, *illyricus*, *Flammula* with its two subspecies (all having 2n = 28

or 32), *R. nivalis* (2n = 56 or possibly 48), *sulphureus* (2n = 96 or ca. 56) and, finally, *R. Lingua* (2n = 128; possibly half this number, too). The hydro- and helo-phytes spread intensively by vegetative means, but also species like *R. hyperboreus* and *illyricus*.

Apomixis occurs in *R. Ficaria*, which is generally seed-sterile in Scandinavia but propagates by bulbils. The bulbiliferous forms are triploid or tetraploid, never diploid. The complex around *R. auricomus* (including *R. cassubicus* and *fallax*) reproduces by agamospermy. The chromosome numbers range from 2n = 16 to 48.

In consequence, the annuals have not exceeded the tetraploid status. The perennials have remained sexual with chromosome numbers from 2n=14 up to 128, or they have turned bulbiliferous and agamospermous with 2n = 16, 24, 32, 40 and 48.

*Papaver*. — The annual species, *P. Rhoeas*, *argemone* and *dubium*, are diploid, tetraploid and possibly hexaploid.

The five perennials classified by NORDHAGEN possess octoploid or decaploid numbers.

*Lepidium* (including *Cardaria*). — The annuals are diploid (*L. campestre*, x = 8) or tetraploid (*L. densiflorum*, possibly *ruderale*). The perennial *L. latifolium* is said to possess 2n = 24. *Lepidium Draba* has 2n = 64.

*Cardamine*. — Annuals are *C. impatiens* and *hirsuta*, both diploid with 2n = 16. *Cardamine flexuosa* is tetraploid with 2n = 32.

The perennial species *C. bellidifolia* and *amara* are diploid, the latter intensively reproducing by means of runners. *Cardamine pratensis*, which has 2n numbers between 30 and 84 (LÖVKVIST, 1947), spreads abundantly by propagules formed on the leaves. It often occurs in seed-sterile condition. The duodecaploid, seed-sterile *C. bulbifera* propagates by numerous bulbils, as well as creeping branched rhizomes.

LÖVKVIST (l. c.) discovered that several chromosome races of *C. pratensis* grow together in the analysed meadows of South-Sweden. They differ, however, in their ecological demands. High parts of the meadows house 30-chromosomed types. Lower parts contain clones with 2n = 56—68. Near or in the water we find the highest chromosome numbers (2n = 72—76), illustrating the influence of hydrophytic conditions.

In consequence, annual species reach the tetraploid status. Perennials may turn apomictic. Simultaneously they acquire high chromosome numbers.

*Sedum*. — The single annual investigated (*S. annuum*) is diploid with 2n = 22. The perennials show 2n = 22 (16?) up to 64. The

North-European form of *S. acre*, which reproduces intensively by vegetative means, is hexaploid with 2n = 48.

*Saxifraga.* — The annual *S. tridactylites* and the biennial *S. adscendens* are diploid (2n = 22).

The diploid perennials possess the basic numbers of 10 (*S. tenuis*) and 13 (*S. aizoides, rivularis* and *oppositifolia*). The last-mentioned two species are said to contain tetraploid representatives, too. The rhizomes of the high-basic species form numerous stems. *Saxifraga aizoides* has a conspicuous, although not very strong, vegetative spreading ability. *Saxifraga tenuis* is a delicate, slender plant, without vegetative reproduction. Formerly it was regarded as a variety or form of the polyploid *S. nivalis*.

The tetraploid perennials consist of *S. Cotyledon, Aizoon, stellaris* with 2n = 28, *S. hirculus* with 2n = 32 and *S. hypnoides* with 2n = 44. Some have runners. *Saxifraga nivalis* contains, according to SKOVSTED (1934) and FLOVIK (1940), 28- and 60-chromosomed types. Its Scandinavian representatives have some slight capacity of vegetative propagation.

Higher Scandinavian numbers are met with in *S. foliolosa* (2n = 56 and 64), *S. cernua* (2n = 50 and ca. 66), both species regularly reproducing by bulbils, furthermore the common *S. granulata* with bulblets at the stem-base (2n = 32, 46—60), *S. groenlandica* (different 2n numbers from 56 up to 80 and 84), and, finally, *S. hieraciifolia* with 2n = 112.

Life-form and chromosome number are connected with one another. Apomixis breaks through in the high-polyploid status.

*Alchemilla* (including *Aphanes*). — One species of subgenus Aphanes (*A. microcarpa*) is sexual and diploid (2n = 16?). The other, *A. arvensis*, is polyploid with 2n = ca. 48 but reproduces agamospermously.

All perennials of subg. Eualchemilla are high-polyploid (up to 2n = 120) and apomictic.

*Medicago.* — The annual or biennial species *M. minima* and *lupulina* are generally diploid, although a tetraploid form has been described in the latter species.

The single native perennial (*M. falcata*) is a tetraploid.

*Trifolium.* — This genus affords good evidence.

Annual diploids with the basic number of 7 consist of *T. micranthum, campestre, aureum, spadiceum, striatum, arvense. Trifolium dubium* is the single tetraploid.

The diploid perennials are in the majority. One (*T. pratense*) has the basic number of 7, the others (*T. hybridum, montanum, fragiferum* and *alpestre*) show x = 8. Vegetative reproduction in *T. fragiferum* goes on rather intensively by means of rooting stems.

Polyploids are *T. repens* (2n = 32) and *T. medium* (2n = 80—100—130). Both species migrate rapidly by vegetative means.

Consequently, the annuals have (1) the low basic number of 7, (2) are on an average less polyploid than the perennials (14 against 29 %), and (3) do not reach such high chromosome levels (28 against 130).

*Vicia.* — All annuals (*V. hirsuta, tetrasperma, villosa, lathyroides, angustifolia*) are diploid with the basic numbers 5, 6 or 7. The perennials are either diploid with x = 6 or 7 (*V. pisiformis, orobus, dumetorum, cassubica, silvatica, sepium*) or are tetraploid (with 2n = 24, *V. tenuifolia*; with 2n = 28, *V. cracca*). Of the last-mentioned species some extra-Scandinavian forms are said to be diploid. Several species, diploid as well as polyploid, migrate rapidly by vegetative means.

*Lathyrus.* — The single annual occurring (*L. sphaericus*) is diploid (x = 7). Most perennials stand on the same level. *Lathyrus palustris* is a hexaploid. Several species have an intense vegetative reproduction.

*Geranium.* — Five annuals are diploid, with 2n = 18 (*G. columbinum*), 20 (*G. lucidum*), 22 (*G. dissectum*), 26 (*G. molle* and *pusillum*). Two species are polyploid (*G. lanuginosum* with 2n = 42 and *G. Robertianum* with 2n = 32 or 56).

All perennials are polyploid, beginning with 2n = 28 (*G. silvaticum, pratense* and *pyrenaicum*), 28 or 56 (*G. palustre*) and 84 (*G. sanguineum*). Some have strong, richly branched rhizomes (for instance, *G. sanguineum*).

*Malva.* — The basic number of this genus is said to be 7. It may possibly be as high as 21, since 2n = 42 is the lowest number ever recorded. The annual *M. neglecta* and *pusilla* have 2n = 42 (the latter also 2n = 72?). The perennials pass from 2n = 42 (*M. moschata, silvestris*) to 84 (*M. alcea*).

*Viola.* — In this genus there occur four different basic numbers (6, 10, 13, 17). All species, except *V. tricolor* and *arvensis*, are perennial. These two annuals are diploid but have x = 13 and 17. Nine perennials are diploid (*V. biflora* — x = 6; *V. odorata, hirta, collina, mirabilis, Reichenbachiana, rupestris, stagnina, uliginosa* — x = 10). Some have an inconspicuous vegetative reproduction. Others like *V. stagnina* reproduce rapidly by runners. Tetraploid species are *V. Riviniana, canina*

(with varying chromosome numbers), *pumila, elatior, epipsila, Selkirkii.* A hexaploid is *V. palustris.* The vegetative reproduction is sometimes very intense (*V. epipsila* and *palustris*).

*Myosotis.* — The karyology of the Scandinavian forms is insufficiently analysed. The 2n numbers, determined on Scandinavian material, are known for three species: *M. stricta* (annual, 2n = 36—40), *M. discolor* (annual, 2n = ca. 60), *M. palustris* (perennial, 2n = 64).

*Lamium.* — Four species are annual (*L. purpureum, amplexicaule, hybridum, moluccellifolium,* the first two being diploid, the two others tetraploid). One perennial appears to be exclusively diploid (*L. album*); another one (*L. Galeobdolon*) contains diploid as well as tetraploid types. Both perennials reproduce intensively by vegetative means.

*Stachys* (including *Betonica*). — One annual, *S. arvensis,* is diploid with the basic number 5. The perennial *S. officinalis* shows the somatic number of 16 (x = 8). *Stachys silvatica* and *palustris* are high-polyploids with 2n = 48, 66 and 2n = ca. 64, 102 respectively. The vegetative multiplication is more or less intense (rhizomes, runners, tuber-like propagules). Such is especially the case in the high-polyploid *S. palustris.*

*Solanum.* — Here we find an apparent exception to the rule that annuals are low-chromosomal. The therophytic species, *S. alatum* and *nigrum,* are polyploid with 2n = 48 and 72 (x = 12) in Scandinavia. Both are weeds. Some varieties of *S. nigrum* from India possess lower numbers (2n = 24 and 48). *Solanum dulcamara* belongs to the phanerophytes. Like most of these it is characterized by a diploid constitution.

*Veronica.* — The annual group of species possesses three different basic numbers (x = 7, 8 and 9). *V. triphylla* and *polita* (2n = 14), *arvensis* (2n = 14 or 16), *verna* (2n = 16) and *praecox* (2n = 18) are diploid. *V. agrestis, opaca* and *persica* are tetraploid and, finally, *V. hederifolia* is hexaploid.

In addition to the idiograms of 7, 8 and 9 the diploid perennials possess the derived x number of 17. Original species are *V. serpyllifolia* (14), *fruticans* (16), *alpina, montana, scutellata* and *Beccabunga* (18). Some have an intense vegetative spreading.

*Veronica longifolia* is said to be tetraploid (2n = 68), its subspecies *maritima,* diploid (2n = 34). Exclusively tetraploid are *V. Chamaedrys* (2n = 32), *officinalis, Anagallis-aquatica, comosa* (2n = 36). Their vegetative dispersal is intense. The polyploids amount to 45 % (the doubtful case of *V. longifolia* excluded) against 38 % in the annual species. Two of three helophytes are tetraploid.

The basic numbers of the Scandinavian species are distributed in the following manner:

| Basic number | 7 | 8 | 9 | 17 |
|---|---|---|---|---|
| No. of annuals ..... | 6—7 | 1—2 | 1 | — = 9 |
| No. of perennials .... | 1 | 2 | 7 | 2 = 12 |

*Plantago.* — One diploid annual is native to Scandinavia (*P. coronopus*, x = 5). The other annual, *P. tenuiflora*, is said to be polyploid (2n = 24).

The wide-spread weedy *P. major* occurs exclusively as diploid (x = 6). In general it does not reproduce vegetatively (is a »stationary weed»). Scandinavian *P. lanceolata* is said to be diploid (x = 6), but polyploid varieties have been described from Bucharest (2n = 24) and Munich (2n = 96). Their true classification has not been elucidated. *Plantago media* appears exclusively as a tetraploid (2n = 24). In contrast to the other species it reproduces freely by vegetative means (small pieces cut off from the mighty tap-root).

According to Mc CULLAGH (1934), the annual *P. coronopus* has the smallest chromatin amount among the species examined, even less than the 8-chromosomed *P. ovata*. (Cf. the conditions within *Crepis* and *Dianthus*; GUSTAFSSON, l. c., p. 294.)

*Galium* (including *Asperula*). — Three species occur as annuals: *G. Vaillantii* (2n = 20), *spurium* (2n = 20, possibly 44) and *Aparine* (ca. 64, ca. 86, also 22?, 44?). The basic numbers are 10 and 11. *Galium Aparine* has followed man since the beginning of civilization.

The perennials show a third basic number (x = 12). One species is recorded as diploid (*G. trifidum*, 2n = 24). All other species contain either several diploid and polyploid chromosome races or are exclusively polyploid. *Galium verum* possesses 2n = 22 and 44 (the first-mentioned number not known from Scandinavia), *G. Mollugo* 2n = 22, 44, 55, 66 (44 in Scandinavia). The principal form of *G. palustre* is diploid (2n = 24) and occurs in moist places, ditches, etc (FAGERLIND, 1937). Its subspecies *elongatum* with 2n = ca. 96 grows directly in water. *Galium uliginosum* is always tetraploid in Scandinavia (2n = 44) but is said also to contain diploid representatives. *Galium triandrum, odoratum, pumilum, hercynicum* are tetraploid (2n = 44). *Galium boreale* is known as tetraploid and hexaploid (x = 11).

The frequent polyploidy corresponds to an intense capacity of vegetative reproduction.

*Campanula.* — The chromosome numbers of the biennial species *C. Cervicaria* and *patula* are unknown. A third biennial, *C. rapunculus*, occurs as semi-spontaneous. It is diploid (2n = 20). In the perennials there is a wide range in chromosome number from 2n = 16 up to 102 (in *C. rapunculoides*, one of the typical »root-wandering weeds»). This as well as some diploid and tetraploid species possess 17 as their basic number.

*Erigeron.* — The single annual, *E. canadense*, is a wide-spread weed with 2n = 18. The four perennials are either diploid (x = 9; *E. acre*, which may appear as biennial, too; *E. boreale* and *uniflorum*) or tetraploid (*E. unalaschkense*).

*Gnaphalium.* — The two annuals, *G. uliginosum* and *luteo-album*, are diploid with 2n = 14. *Gnaphalium norvegicum* and *silvaticum* are perennial and possess high chromosome numbers (2n = 56 and 58—60). The last-mentioned species reproduces freely by vegetative means.

*Chrysanthemum* (including *Tanacetum*). — *C. segetum* is annual and diploid (2n = 18). One perennial (*C. vulgare*) has the diploid number. It easily reproduces by vegetative means. Tetraploids are *C. corymbosum* and *Leucanthemum*. A decaploid species is *C. arcticum*, according to Japanese determinations. The common ox-eye daisy (*C. Leucanthemum*) propagates by short runners from the woody root-stock. It is a persistent, vigorous weed in old meadows, in Europe as well as in North-America.

*Senecio.* — Five annuals are either diploid (*S. vernalis*) or tetraploid (*S. vulgaris, viscosus, silvaticus*, with 2n = 40, and *S. congestus* with 2n = 48). All Scandinavian perennials investigated are tetraploid (*S. erucifolius, Jacobaea, paludosus, nemorensis* with 2n = 40).

*Cirsium.* — The diploid *C. palustre* with 2n = 34 and the tetraploid *C. vulgare* with 2n = 68 are biennial species. The perennial species certainly behave as diploids, but their high basic number argues for a derived position. The vegetative reproduction is extremely intense in *C. heterophyllum* and *arvense* and proceeds with the aid of long creeping root-stocks and runners.

*Centaurea.* — *C. Cyanus* is annual and diploid (2n = 24). The perennial species *C. Scabiosa* and *phrygia* possess 2n = 20 and 22. Tetraploid species are *C. nigra* and *Jacea*. The last-mentioned species, which is original in Scandinavia, possesses a certain capacity of vegetative reproduction.

*Crepis.* — The single annual (*C. capillaris*) is a diploid with x = 3.

The three biennial species are either diploid (*C. nicaeensis* and *tectorum*, x = 4) or polyploid (*C. biennis*, 2n = 40).

The perennials are diploid with x = 4, 5 or 6 (*C. praemorsa*, *multicaulis*, *sibirica*, *paludosa*).

*Sonchus*. — Both annual species are diploid (*S. oleraceus*, x = 8; *S. asper*, x = 9). Similarly, one perennial is diploid (*S. palustris*, x = 9). The sow-thistle, *S. arvensis*, wide-spread as an aggressive weed, has a high chromosome number (2n = 64) and spreads copiously by vegetative means.

The following table visualizes the chromosome conditions within the thirty-seven Scandinavian genera examined (*Myosotis* excluded):

| Chromosome status | 2x | 4x | 6x | 8x | ≧10x |
|---|---|---|---|---|---|
| No. of annual groups | 12 | 10—12 | 3—7 | 3—5 | 0— 1 = 28—37 |
| No. of perennial groups | 3 | 8 | 1 | 7 | 16—18 = 35—37 |

There appears to be no doubt that in general the annual species keep to the diploid or tetraploid state. Some of the high-polyploid groups are insufficiently analysed, or the polyploid numbers are determined on foreign material (*Juncus bufonius* var. *ranarius*, *Chenopodium album*, the *Cerastium* species, *Medicago lupulina*, *Geranium Robertianum*, *Malva neglecta* and *pusilla*, *Plantago tenuiflora*).

Annual and biennial species, with a fair degree of certainty reaching the hexaploid status, consist of *Bromus commutatus*, *Juncus bufonius*, *Polygonum aviculare*, *Papaver argemone* and *dubium*, *Alchemilla arvensis*, *Solanum nigrum*, *Veronica hederifolia*, *Galium Aparine*, *Crepis biennis*.

*Alchemilla arvensis* occupies a special position, since it has passed over to agamospermy and, furthermore, spreads anthropochorously. This species follows the general rule concerning the combination of apomixis and polyploidy. In conformity with *Polygonum aviculare*, the *Papaver* species and *Galium Aparine*, it is grouped as an »archaeophyte» by BUCHLI (1936), a term covering such species as cannot be regarded as spontaneous anywhere (?) but have been the steady companions of man since prehistorical times. *Solanum nigrum* is ranked as an »ergasiophygophyte», a medicinal plant of foreign extraction, which without man's intention escaped into half-cultivated soil. *Veronica hederifolia* and *Crepis biennis* appear in North-Europe as »epocophytes» (?) and *Juncus bufonius* as a »spontaneous apophyte». Neglecting this complex terminology, we may express the situation thus, that these species have followed man for long periods of time. Human influence has

worked upon them continually. In many anthropochorous groups of species, the original diploid populations were apparently unable to create special ecotypes speedily enough, or the ecotypes could not compete with the products formed by amphiploidy and autoploidy, fit for man's society but scarcely adapted to original habitats.

Three Scandinavian genera contain perennials that keep to the diploid status, viz. *Solanum*, *Cirsium*, *Crepis*.

The single perennial species of *Solanum* (*S. dulcamara*) belongs to the (nano-)phanerophytes. These generally remain in the diploid state. In this special case the basic number is rather high ($x = 12$). The cultivated *Solanum tuberosum*, with its pronounced mode of vegetative propagation, is a polyploid with $2n = 48$.

The *Cirsium* perennials possess the derived basic number of 17. Such high numbers, involving a disguised polyploidy, often act on the vegetative parts in a way similar to direct polyploidy.

With regard to *Crepis*, finally, BABCOCK and JENKINS (1943) point out that the primitive species of this genus are perennial. The most advanced species consist of precocious annuals. These have a very low basic number and, in addition, a low chromatin content.

Anyhow, no more than 20 or at most 35 % of the annual groups have been able to exceed the tetraploid level, against 70 % of the perennial groups. This contrast (involving a P value of 0,001 or less) is founded, I think, on the biological consequences following annuality and perennity.

## VI. CONCLUSIONS.

The facts presented here strengthen the view that the chromosome conditions of the phanerogam species correspond to life-form, growth habit, vegetative reproduction in a remarkable way. Many complex genera, displaying annuality, perennity, intense dispersal by vegetative means, apomixis, build up their cytological properties according to definite rules: Annual groups show low basic numbers and keep to the diploid or low-polyploid state. Perennial groups, still being sexual, often acquire high basic numbers and reach high-polyploid levels. Apomictic groups form the top of the cytological system, polyploidy proceeding further upwards.

Self-incompatibility and vegetative propagation are thought to be two important agents in these evolutionary events. Clone-like spreading in self-sterile species favours the function of unreduced gametes. Poly-

ploidy, in its turn, enhances an already existing asexual reproduction or may, incidentally, induce it. Autogamy, on the other hand, counteracts the function of unreduced gametes. It delays recombination and prevents polyploid stabilization if a polyploid has been able to form. The life-form system, working together with the type of fertilization, helps to change the species in various directions, pass it over to the compressed state of polyploidy, or hold it in the transformable state of diploidy.

The plant species now existing are end-links in a long series of bygone populations. They are sorted out according to their edaphical and climatical demands. They differentiate into ecotypes or form new (eco)species by different means. Corresponding to the exterior factors there arise fundamental changes in physiology, mode of growth, life-form. This led RAUNKIAER to advance his conceptions of therophyte, chamaephyte, hemi-cryptophyte and phanerophyte climates and floras. This paper further emphasizes the fact that the phanerogam species develop and transform according to evolutionary rules which differ from those prevailing in all higher animal groups.

Important results of this and the preceding paper may be summarized in the following way:

*Annual plants* largely remain in the diploid state or, if they turn polyploid, do not easily exceed the tetraploid level. Annuality is often combined with an active decrease in basic number and a low chromatin content. In addition, autogamy frequently prevails.

*Perennial plants* often consist of polyploids. In several genera the perennial diploids have acquired high basic numbers. Hydro- and helophytes, as well as other species propagating intensively by vegetative means, are made up of polyploids (or derived diploids) to a very high extent. Many occur clone-like in nature and do not generally form seeds. They have become apomictic.

*Agamospermous plants*, as well as viviparous apomicts of numerous genera, almost exclusively consist of polyploids. Some retain a certain ability of sexual seed formation. In other instances the sexual faculties are entirely extinguished. Self-sterility predominates in the sexual relatives of most agamospermous species.

*Arctic floras* certainly contain a high proportion of polyploids. This is not due, however, to the direct effects of extreme temperatures on sex-cell formation. Rather it is caused by changes in the life-form system, reacting to the climate by an increase in vegetative dispersal, bulbilifery and agamospermy. These changes facilitate the establish-

ment of apomixis and polyploidy. The sexual representatives are about as polyploid as average Scandinavian mountain species.

*Alpine floras* contain a certain, although not very high, percentage of apomictic species. The most common species are diploid. The average rate of polyploidy is not specially high. An analysis of the snow-layer flora in the Dovre massif indicates that the severe climate does not necessarily induce high-polyploidy. Indeed, the alpine floras are less polyploid than beach and shore floras.

*Halophyte conditions* do not favour the polyploid status. The halophytes of Roumania are predominantly diploid. This corresponds to a high proportion of annual species. The number of perennial halophytes increases considerably in North Germany. As a consequence, the rate of polyploidy augments. This parallelism is especially striking in species having a northern distribution.

*The shore floras* display a derived chromosome status. This is owing to the hydrophytic species, which generally appear as polyploid (or high-basic). The polyploids occupy great parts of the shore areas, consequent upon an exaggeration of the vegetative system, following polyploidy and permitting a dispersal by rhizomes, stolons and runners.

*The weed floras* consist of numerous seed-reproducing annuals, which have not passed the diploid state; of »stationary» perennials, which are slightly more polyploid; and, finally, of »root-wandering» perennials, in which polyploidy predominates. Some root-wandering polyploids have turned into highly aggressive and persistent weeds. Man's society apparently favours polyploidy. This becomes evident from the analysis of hemerophilous and hemerophobous elements within one and the same flora region. Culture conditions contribute, I think, to the change of annual diploids into polyploid weeds. Presumably the diploid predecessors were not able to create successful (agro-)ecotypes, or these could not compete with the autoploid and amphiploid products.

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*Addendum.* — Recently BÖCHER (in Kgl. Danske Videnskab. Selsk. 20, No. 8, 1947) has shown that the Scandinavian representatives of *Geranium Robertianum* contain $2n = 64$. Some South-European forms have $2n = 32$. The number $2n = 56$ does not occur.

# GENETIC VARIATION IN THE FREQUENCY OF MULTIPLE BIRTHS IN CATTLE

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## INTRODUCTION.

IN connection with an investigation of some causes influencing the fertility of cows the author collected information concerning a number of Ayrshire herds in southern Finland, including data on the frequency of multiple births in these herds (KORKMAN, 1946). As, however, this material in itself was not very extensive, it was not used for investigations on the heredity of multiple births in cattle. Later on, through the compliance of Professor IVAR JOHANSSON of the Institute of Animal Breeding, the author was granted use of the material on which the paper »The sex ratio and multiple births in cattle» (JOHANSSON, 1932) was founded and it was then possible to study this problem more closely.

The purpose of the present investigation was primarily to elucidate whether and to what extent the frequency of multiple births in cattle is influenced by the individuality of the animals. In this connection the question whether special causes influence the frequency of monozygotic and dizygotic twinning has also been dealt with.

## MATERIAL AND METHOD.

The part of the material which was collected by the author, comprises altogether 13.119 births within 38 Ayrshire herds in southern Finland during the years 1931—1937. As the material was originally collected for another purpose, certain demands were made of the herds for their inclusion in the investigation. Only such herds were included as had been free from tuberculosis and contagious abortion. The average number of cows in the herds during the years in question was at least 30. Further, only such herds were considered in which feeding and production had not varied to any great extent from one year to another. During personal visits to the farms records were made of the date of birth and parents of each cow, of all calvings during the period

in question as well as of single or multiple births. The sex of the calves was, however, not recorded.

The second part of the material comprises altogether 11.839 calvings in Swedish Friesian herds and 53.703 calvings in herds of Swedish red and white cattle. Data on these calvings were collected from the farms in question with the intention of investigating the cause of multiple births. Therefore, they give detailed information about each calving, including the sex of the calves. When possible, data about the date of birth and parents of the cows as well as of the sire were collected. These data are not restricted to any definitely limited period but include information of calvings from the end of the 19th century up to the late 1920's.

The methods according to which both the Finnish and the Swedish material were collected do not imply any selection with regard to the frequency of multiple birth. Therefore the material may be considered typical of the breeds within the respective areas at the time from which the material dates.

The statistical treatment of the material is based on common principles as expressed by SNEDECOR (1938) and BONNIER and TEDIN (1940) among others. Division of the mean squares into their theoretical components was made according to the method developed by WINSOR and CLARKE (1940).

## ANALYSIS OF SOME OF THE CAUSES OF VARIATIONS IN THE FREQUENCY OF MULTIPLE BIRTHS.

The analysis of the variations in the frequency of multiple births is founded on the occurrence of two variables, single birth indicated 0 and multiple birth indicated 1. Earlier investigations, carried out on cattle as well as on several other animal species and in man, have shown that the age of the mother influences the frequency of multiple births (JONES and ROUSE, 1920; BONNEVIE and SVERDRUP, 1926; DAHLBERG, 1926; HEWITT, 1934; WEINBERG, 1928, etc.). As this has been proved also to apply to the material presented here (JOHANSSON, 1932; KORKMAN, 1946), the variation caused hereby was principally eliminated by the fact that in the Finnish material, which comprises calvings during a limited number of years, the cows were divided into groups each including cows born within three successive years. In the Swedish part of the material the cows were divided into groups with equal number of calvings per cow within the groups. As it has further been asserted

that actual environmental factors also influence the frequency of multiple births (e. g. JOHANSSON, 1932; LENZ, 1933), the variations within the herds were investigated. Table 1 shows how the method developed by WINSOR and CLARKE (1940) was applied to the Finnish material.

TABLE 1. *Analysis of the variation in frequency of multiple births in Finnish Ayrshire cattle.*

| Cause of variance | Degrees of freedom | Mean square $\times 10^4$ | Theoretical components of the mean square | Calculated variance | |
|---|---|---|---|---|---|
| | | | | $\sigma^2 \times 10^4$ | percentage of the total variance |
| Between herds and between age groups... | p — 1 = 240 | 312,865 [3] | $A+(m/n)B+$ $+(m/o)C+$ $+(m/p)D$ | D = 1,081 | 0,70 |
| Between sires of the cows | o — p = 633 | 254,019 [1] | $A+(m/n)B+$ $+(m/o)C$ | C = 6,507 | 4,19 |
| Between cows | n — o = 3135 | 156,354 [2] | $A+(m/n)B$ | B = 3,794 | 2,44 |
| Within cows | m — n = 9110 | 143,939 | A | A = 143,939 | 92,67 |
| Total variance | m — 1 = 13118 | 155,309 | $T = A+$ $+B+C+D$ | T = 155,321 | 100,00 |

[1] $P < 0{,}001$. — [2] $P < 0{,}01$. — [3] $P < 0{,}05$.

m = total number of births = 13.119
n = » » » cows = 4.009
o = » » » sires of the cows within the age groups = 874
p = » » » age groups within the herds = 241

A = $\sigma^2$ (variance) caused by difference between the births in the same cow,
B = $\sigma^2$ caused by differences between the daughters of the same sire within the age groups and herds,
C = $\sigma^2$ caused by differences between the sires within age groups and herds,
D = $\sigma^2$ caused by differences between herds and age groups,
T = $\sigma^2$ total variance.

It appears from this investigation (Table 1) that differences between the sires influenced the frequency of multiple births and also that there are differences between paternal half-sisters. The variance caused by differences between the sires is 4,2 per cent and that depending on differences between paternal half-sisters is 2,4 per cent of the total variance.

TABLE 2. *Analysis of the variation in frequency of multiple births in Swedish Friesian cattle.*

| Cause of variance | Degrees of freedom | Mean square $\times 10^4$ | Calculated variance | |
|---|---|---|---|---|
| | | | $\sigma^2 \times 10^4$ | percentage of the total variance |
| Between herds and between groups of cows with equal number of calvings within the groups | 449 | 475,814 [4] | 0,441 | 0,13 |
| Between sires of cows | 712 | 465,831 [2] | 10,212 | 3,01 |
| Between cows | 938 | 376,196 [2] | 12,183 | 3,58 |
| Within cows | 8091 | 317,073 | 317,073 | 93,28 |
| Total variance | 10190 | 339,895 | 339,909 | 100,00 |

[2] $P < 0{,}01$. — [4] $P > 0{,}05$.

Table 2 shows the result of the corresponding analysis of the data from Swedish Friesian herds. As data on the descent of the cows were missing in some cases, this analysis comprises only 10.191 births out of the total number of 11.839.

There are also in this case significant differences between the progeny groups of different sires, and between the cows in these groups. The variance caused by differences between the sires is here 3,0 per cent, and that due to the differences between paternal half-sisters is 3,6 per cent of the total variance.

TABLE 3. *Analysis of the variation in frequency of multiple births in Swedish red and white cattle.*

| Cause of variance | Degrees of freedom | Mean square $\times 10^4$ | Calculated variance | |
|---|---|---|---|---|
| | | | $\sigma^2 \times 10^4$ | percentage of the total variance |
| Between herds and between groups of cows with equal number of calvings within the groups | 310 | 237,806 [1] | 0,678 | 0,40 |
| Between sires of cows | 1739 | 162,785 [4] | — | — |
| Between cows | 2693 | 192,265 [1] | 7,313 | 4,35 |
| Within cows | 16160 | 160,034 | 160,034 | 95,25 |
| Total variance | 20902 | 165,569 | 168,025 | 100,00 |

[1] $P < 0{,}001$. — [4] $P > 0{,}05$.

Finally, the data from the Swedish red and white herds were also analysed in the same way in Table 3. This analysis does not show any important difference between the progeny groups of different sires, whereas there is a significant difference between the cows of these groups. The variance due to the difference between paternal half-sisters is 4,4 per cent of the total variance.

Based on the results of these analyses the correlation between the frequency of multiple births for different calvings of the same cow was also calculated. This intra-cow correlation or repeatability coefficient ($r_i$) was calculated according to the following formula: $r_i = \frac{B+C}{A+B+C}$ with $A = \sigma^2$ within cows, $B = \sigma^2$ between cows with the same sire and $C = \sigma^2$ between the sires. The result is as follows:

Finnish Ayrshire = 0,067
Swedish Friesian = 0,066
Swedish red and white = 0,044.

It should be pointed out that the group division in the analysis of the Finnish Ayrshire cattle does not completely correspond to that in the other two analyses. In the first the grouping is arranged so that each age group actually comprises only cows born within the same year, but, as the material comprises data from totally 6 record years and a considerable number of the animals were rejected during this period, the different age classes will include cows with a different number of calvings. As a consequence, part of the variation caused by the different ages of the animals also appears in the subgroups. Owing to this fact the variation between the sires will in all probability increase and the figure 4,19 per cent be somewhat too high. Likewise the intra-cow correlation coefficient is a little too high.

In the two analyses having reference to the Swedish material the grouping was arranged so that cows with the same number of calvings were put together into groups. While in the first analysis each cow had 6 calvings at the most, the latter includes groups with as many as 11 or more calvings per cow. Consequently, in this latter case a greater part of the variance caused by differences of age is found in the variance within cows than in the former case. The variance within cows is therefore comparatively larger in analyses 2 and 3 than in analysis 1, a fact which reduces the remaining variance portions and the intra-cow correlation.

## CORRELATION BETWEEN DAMS AND DAUGHTERS AND BETWEEN FULL SISTERS.

Comparison of the frequency of multiple births between dams and their daughters was made in such manner that the result of all calvings, except the two first, were considered both for the dams and for their daughters. As before in this investigation, every single birth was here given the value 0 and every multiple birth the value 1. In this way all dam-daughter pairs from the Swedish Friesian herds were investigated whereas only such Swedish red and white herds as had records for at least 25 cows were included. As the data concerning Finnish Ayrshire herds provide calving records for at most 6 successive years, this part of the material was not included. In this comparison the total dam-daughter correlation was calculated both for the total and within sires.

TABLE 4. *Dam-daughter and full sister correlation in the frequency of multiple birth.*

| Cause of variance | Swedish Friesian cattle | | | Swedish red and white cattle | | |
|---|---|---|---|---|---|---|
| | Degrees of freedom | Coefficient of correlation | P-value | Degrees of freedom | Coefficient of correlation | P-value |
| Total dam-daughter correlation ..... | 444 | 0,088 | $< 0{,}1$ | 1391 | $-0{,}021$ | $< 0{,}5$ |
| Dam-daughter correlation within sires ..... ..... | 251 | $-0{,}036$ | $< 0{,}6$ | 1094 | 0,001 | $> 0{,}9$ |
| Correlation between full sisters ..... | 113 | 0,080 | $< 0{,}4$ | 252 | 0,109 | $< 0{,}1$ |

The comparison of the frequency of multiple births in full sisters was performed according to the same principles and within the same part of the material as that in dams and their daughters. In this case, however, only the total correlation was calculated. The result is shown in Table 4. It appears that none of the correlation coefficients is significant.

## RELATION BETWEEN LIKE-SEXED AND UNLIKE-SEXED TWINS.

The investigation also includes an analysis of the causes of variations in twin births both in cases of twins of the same sex and of different

sex. Thus, the first group also includes any monozygous twins that may occur. This analysis was, however, only made on the data from Swedish Friesian herds, where the highest frequency of twin births was found. The result appears from Table 5.

TABLE 5. *Analysis of the variation in frequency of birth of like-sexed and unlike-sexed twins in Swedish Friesian cattle.*

| Cause of variance | Degrees of freedom | Variation in the frequency of like-sexed twins | | | Variation in the frequency of unlike-sexed twins | | |
|---|---|---|---|---|---|---|---|
| | | Mean square $\times 10^4$ | Calculated variance | | Mean square $\times 10^4$ | Calculated variance | |
| | | | $\sigma^2 \times 10^4$ | percentage of the total variance | | $\sigma^2 \times 10^4$ | percentage of the total variance |
| Between herds and between groups of cows with equal number of calvings within the groups ... | 449 | 229,352 [1] | 2,503 | 1,49 | 216,264 [2] | 1,654 | 1,02 |
| Between cows | 1650 | 172,673 [4] | 1,841 | 1,10 | 178,803 [1] | 4,750 | 2,93 |
| Within cows | 8091 | 163,441 | 163,441 | 97,41 | 155,751 | 155,751 | 96,05 |
| Total variance | 10190 | 167,840 | 167,785 | 100,00 | 162,150 | 162,155 | 100,00 |

[1] $P < 0{,}001$. — [2] $P < 0{,}01$. — [4] $P > 0{,}05$.

It appears that the variance which may be considered as caused by genetic factors, viz. in this case the variance between cows within herds and within groups of cows with equal number of calvings, is larger for unlike-sexed than for like-sexed twins. In the former case the variance ($\sigma^2$) is 0,0004750, in the latter only 0,0001841. The difference between the corresponding mean squares is not significant, however, and therefore no general conclusions would be justified.

On the basis of the same material JOHANSSON (1932) showed that the month of calving influences the frequency of multiple births. In order to investigate whether the influence of the month of calving is the same with regard to both like-sexed and unlike-sexed twins, a comparison was made in Table 6 showing their frequency during different months of calving. As will be seen, even a superficial examination shows that the frequency of unlike-sexed twins is evener for the various

TABLE 6. *The frequency of multiple births and the frequency of birth of like-sexed and unlike-sexed twins during various months.*

| Month of calving | Swedish Friesian cattle | | | | | | | Swedish red and white cattle | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of calvings | multiple births | | like-sexed | | unlike-sexed | | Number of calvings | multiple births | | like-sexed | | unlike-sexed | |
| | | Number | % | Number | % | Number | % | | Number | % | Number | % | Number | % |
| I | 1118 | 49 | 4,38 | 28 | 2,50 | 21 | 1,88 | 5192 | 101 | 1,95 | 47 | 0,91 | 49 | 0,94 |
| II | 934 | 29 | 3,10 | 10 | 1,07 | 17 | 1,82 | 4381 | 82 | 1,87 | 40 | 0,91 | 38 | 0,87 |
| III | 821 | 24 | 2,92 | 10 | 1,22 | 13 | 1,58 | 4347 | 76 | 1,75 | 32 | 0,74 | 37 | 0,85 |
| IV | 701 | 18 | 2,57 | 10 | 1,43 | 7 | 1,00 | 4212 | 80 | 1,90 | 45 | 1,07 | 34 | 0,81 |
| V | 703 | 29 | 4,13 | 12 | 1,71 | 13 | 1,99 | 3793 | 81 | 2,14 | 42 | 1,11 | 32 | 0,84 |
| VI | 692 | 34 | 4,91 | 20 | 2,89 | 11 | 1,59 | 3688 | 78 | 2,11 | 39 | 1,06 | 28 | 0,76 |
| VII | 959 | 34 | 3,55 | 16 | 1,67 | 17 | 1,77 | 3782 | 80 | 2,12 | 40 | 1,06 | 36 | 0,95 |
| VIII | 1061 | 27 | 2,54 | 14 | 1,32 | 11 | 1,04 | 4566 | 95 | 2,08 | 48 | 1,05 | 41 | 0,90 |
| IX | 1139 | 27 | 2,37 | 10 | 0,88 | 17 | 1,49 | 4758 | 82 | 1,72 | 38 | 0,80 | 38 | 0,80 |
| X | 1341 | 45 | 3,36 | 18 | 1,34 | 20 | 1,49 | 4678 | 68 | 1,45 | 39 | 0,83 | 26 | 0,56 |
| XI | 1181 | 40 | 3,39 | 18 | 1,52 | 17 | 1,44 | 5040 | 77 | 1,53 | 35 | 0,69 | 32 | 0,64 |
| XII | 1189 | 42 | 3,53 | 22 | 1,85 | 18 | 1,51 | 5266 | 94 | 1,79 | 48 | 0,91 | 38 | 0,72 |

months than that of like-sexed twins. The question whether there is a correlation between the value of the total frequency of twin births during various months and the difference between the frequency of like-sexed and unlike-sexed twin births for the corresponding months, was examined in Table 7.

TABLE 7. *Correlation between the total twinning frequency for various months* ($x$) *and the difference between the frequency of like-sexed and unlike-sexed twins during the corresponding months* ($y$).

| Cause of variance | Degrees of freedom | Sum of squares | | Sum of products | Coefficient of correlation |
|---|---|---|---|---|---|
| | | x | y | | |
| Total ..... | 23 | 21,3075 | 3,7977 | 2,3663 | |
| Between breeds | 1 | 13,7680 | 0,0216 | — 0,5498 | |
| Within breeds... | 22 | 7,5395 | 3,7761 | 2,9161 | 0,547 [2] |

[2] $P < 0{,}01$.

It appears that the greater the twin frequency for a certain month, the greater the number of like-sexed twins in relation to that of unlike-

sexed. The environmental differences thus seem in the first place to influence the frequency of like-sexed twins, resulting in turn in an increased total frequency of twin births.

TABLE 8. *Frequency of multiple births and frequency of like-sexed and unlike-sexed twins in calvings of different order.*

| Order of calving | Swedish Friesian cattle | | | | | | | Swedish red and white cattle | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of calvings | multiple births | | like-sexed | | unlike-sexed | | Number of calvings | multiple births | | like-sexed | | unlike-sexed | |
| | | Number | % | Number | % | Number | % | | Number | % | Number | % | Number | % |
| 1 | 2256 | 23 | 1,02 | 10 | 0,44 | 13 | 0,57 | 10636 | 75 | 0,71 | 37 | 0,35 | 33 | 0,31 |
| 2 | 2170 | 71 | 3,27 | 34 | 1,57 | 31 | 1,42 | 10132 | 177 | 1,75 | 87 | 0,86 | 74 | 0,73 |
| 3 | 1999 | 65 | 3,25 | 29 | 1,45 | 30 | 1,50 | 9028 | 175 | 1,94 | 87 | 0,96 | 71 | 0,79 |
| 4 | 1691 | 78 | 4,61 | 33 | 1,95 | 40 | 2,37 | 7305 | 176 | 2,41 | 88 | 1,20 | 75 | 1,03 |
| 5 | 1310 | 60 | 4,58 | 27 | 2,06 | 30 | 2,29 | 5614 | 128 | 2,28 | 72 | 1,28 | 51 | 0,91 |
| 6 | 952 | 43 | 4,52 | 21 | 2,21 | 17 | 1,79 | 4109 | 102 | 2,48 | 47 | 1,14 | 51 | 1,24 |
| 7 | 636 | 25 | 3,93 | 14 | 2,20 | 10 | 1,57 | 2844 | 75 | 2,64 | 38 | 1,34 | 35 | 1,23 |
| 8 | 393 | 19 | 4,84 | 12 | 3,05 | 6 | 1,53 | 1814 | 38 | 2,09 | 17 | 0,94 | 16 | 0,88 |
| 9 | 228 | 8 | 3,51 | 5 | 2,19 | 3 | 1,31 | 1086 | 21 | 1,93 | 9 | 0,83 | 11 | 1,01 |
| 10 | 115 | 4 | 3,48 | 2 | 1,74 | 2 | 1,74 | 617 | 14 | 2,27 | 7 | 1,13 | 5 | 0,81 |
| 11 | 89 | 2 | 2,25 | 1 | 1,12 | 0 | 0,00 | 518 | 13 | 2,51 | 4 | 0,77 | 7 | 1,35 |

In order to throw light on the question whether the relation between the frequencies of like-sexed and unlike-sexed twins changes with the age of the cows, the material was correlated as in Table 8. In Swedish Friesian cattle the difference between the frequency of like-sexed and unlike-sexed twins appears to be largest between the sixth and the ninth calving, whereas this is not the case in Swedish red and white cattle. No significant correlation between the order of the calving and the difference between the frequency of like-sexed and of unlike-sexed twins can be established when the correlation is calculated within breeds.

## DISCUSSION.

The investigation has shown that cows have a different predisposition to twin births. The established intra-cow correlations, viz. 0,067 for Finnish Ayrshire cattle, 0,066 for Swedish Friesian and 0,044 for Swedish red and white cattle, may best be compared with the values

earlier calculated by JOHANSSON (1932) for the recurrence within cows after one and two twin births respectively. JOHANSSON found that in Swedish Friesians the repeatability after the first twin birth was 12,69 per cent and after two twin births 21,52 per cent, the corresponding percentage for Swedish red and white breed being 6,61 and 16,81. For Württemberg cattle RICHTER (1926) found that the repeatability after the first twin birth was 12,5 per cent. The fact that the repeatability after one or two earlier twin births respectively is greater than the intra-cow correlation may possibly depend on the presence of a predisposition of varying degree in cows. This would be in accordance with JOHANSSON's (1932) opinion on the cause of the difference between the repeatability after one or two twin births. It may also partly be explained by the fact that cows which have previously given birth to twins have already reached an age in which the frequency of twin births is greater. Several authors (e. g. JOHANSSON, 1932; HEWITT, 1934; WEBER, 1944; KORKMAN, 1946) have proved that the frequency of twin births increases with the age of the animals. This is true at least as far as to the ninth calving.

In this investigation significant differences in the frequency of twin births have been established between the daughter groups of different sires. No evident dam-daughter correlation was, however, established, and the value of the non-significant correlation coefficients is almost 0. Neither was any significant correlation between full sisters established. The numerical values of the coefficients are, however, greater than those for the dam-daughter correlation.

All these observations correspond well with observations previously made on the predisposition to twin births in cattle. Thus, JOHANSSON (1932), HEWITT (1934), LÖWE (1939), KARWETZKI (1941), WEBER (1944) and others have come to the conclusion that there must be a hereditary predisposition to multiple births. The difference found in this investigation between the daughter groups of different sires proves that this is the case. Several authors (e. g. LÖWE, 1939; KARWETZKI, 1941; WEBER, 1944) consider it probable that the hereditary predisposition is recessive. This assumption is also supported by the results obtained by the present writer. The fact that no dam-daughter correlation was established may be best explained by the assumption that a recessive heredity exists.

In our material the variation between cows is greater in the birth of unlike-sexed than of like-sexed twins, although the difference is not significant. If, in later investigations, a real difference is found, the

explanation would be that the differences between cows are principally due to the birth of dizygotic twins. Such an assumption would seem to be supported by already published data on the frequency of monozygotic twins in different breeds. Thus WEBER (1944) found in the Simmental breed a total frequency of twin births of 4,6 per cent and estimated 1,72 per cent of the total number of twins to be monozygotic. BONNIER (1946) estimated, on the other hand, that of all like-sexed twins 4,83 per cent in the Swedish Friesian breed and 10,10 per cent in the Swedish red and white cattle were monozygotic. According to JOHANSSON (1932), the total frequency of twin births is 3,35 per cent in the Friesian cattle and 1,85 per cent in the red and white cattle. According to WARD (1946), the frequency of twinning in New Zealand herds is 0,99 per cent. On 117 pairs of unlike-sexed twins came 145 pairs of like-sexed twins; the 26 pairs of like-sexed twins in excess may be assumed to be monozygotic. If on the basis of these figures the total number of monozygotic twins is calculated in per cent of the total number of births, the following result is obtained:

| | | |
|---|---|---|
| Simmental cattle | 0,08 | per cent |
| Swedish Friesian cattle | 0,08 | » » |
| Swedish red and white cattle | 0,09 | » » |
| New Zealand herds | 0,09 | » » |

The differences between breeds thus seem to apply mainly to the birth of dizygotic twins.

DAHLBERG (1926) proved that in man the frequency of monozygotic twins is almost independent of the age of the mother, whereas an evident age curve can be established for the frequency of dizygotic twins. The present author's investigation does not support the assumption that this would be the case with cattle.

## CONCLUSIONS.

The most important results of this investigation are:

(1) The intra-cow correlation for the frequency of multiple births is 0,067 for Finnish Ayrshire cattle, 0,066 for Swedish Friesian cattle, and 0,044 for Swedish red and white cattle.

(2) Significant differences in the frequency of multiple births were established between groups of daughters of different sires.

(3) No relation between the frequency of multiple births in dams and daughters could be found.

(4) The difference between breeds as to the frequency of unlike-sexed twins seems to be larger than that of like-sexed twins.

(5) The environmental differences in the frequency of multiple births between different months of calving would seem to depend principally on a different frequency of like-sexed twins.

(6) No relation between the order of calving and the difference between the frequencies of like-sexed and unlike-sexed twins could be established.

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# BEHAVIOUR OF ACCESSORY RYE CHROMOSOMES IN THE EMBRYO-SAC

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## INTRODUCTION.

IT is known since 1924 (GOTOH, KIHARA) or even since 1911 (NAGAO) that certain rye plants have sixteen chromosomes. The morphology and behaviour of the additional chromosomes have been studied by several investigators, especially by MÜNTZING (MÜNTZING, 1943, 1944, 1945, 1946; MÜNTZING and PRAKKEN, 1941). The genetic and cytological studies of MÜNTZING were made on strains derived from a plant with 16 chromosomes in »Östgöta Gråråg» and from a similar plant in »Vasa II». The additional rye chromosomes are often called fragment chromosomes (fr.) or B-chromosomes, and they belong to a class of chromosomes that has been termed accessory chromosomes (MÜNTZING, 1945; HÅKANSSON, 1945).

It is necessary to call attention to some results of the investigations of MÜNTZING. The standard fragment in »Östgöta Gråråg» has a subterminal centromere and is smaller than the ordinary chromosomes (= the A-chromosomes). It shows reduced pairing, in plants with two standard fragments they are unpaired in 61,8 % of the p. m. c's. The fragments of »Vasa II» are somewhat larger and show much better pairing, being unpaired in only 11,1 % of the p. m. c's. An unpaired fragment usually divides at first anaphase, and is not infrequently eliminated at second anaphase. Very strange is the behaviour of accessory rye chromosomes at the first pollen mitosis. In 1934 HASEGAWA found that the fragment chromosome, as a rule, is subject to non-disjunction, with the result that the generative nucleus of most pollen grains has two fragments, the tube nucleus no fragment. MÜNTZING studied this non-disjunction more closely: at early anaphase the standard fragment lags between the anaphase groups and has the appearance of a bivalent with a chiasma on each side of the centromere. »This structure arises because the centromere has divided and tries to pull the two chromatids apart. Resistance to this separation is offered in a special, probably heterochromatic segment of the long arm not far from the centromere» (MÜNTZING, 1946). The unseparated chromatids then pass

to the generative pole. The cause of non-disjunction is not a defect of the centromere of the fragment, as has been suggested by DARLINGTON, but a delayed reproduction or stickiness in the special segment. Two new fragment types have arisen, probably through misdivision of the centromere of the standard fragment. One of them is a large iso-chromosome lacking the short arm but having the long arm duplicated, the other is a small iso-chromosome lacking the long arm and having the short arm duplicated. In plants with the large iso-chromosome non-disjunction of this chromosome was observed at the pollen mitosis. However, the small iso-chromosome shows a different behaviour, it divides normally and its chromatids pass to opposite poles.

The crossing experiments of MÜNTZING, as well as those of Russian and British investigators, have shown that in the offspring of rye plants with fragment chromosomes or in crosses of such plants with normal plants one can only rarely find a plant having an odd number of fragments. This may be explained by the non-disjunction of the accessories at the pollen mitosis. However, crosses of the type fr.-plant ♀ × normal ♂ also result in plants with an even number of chromosomes; only rarely has the functioning egg-cell an odd number of fragments, and therefore it is concluded that a directed non-disjunction occurs on the female side too.

ÖSTERGREN (1947) studied the behaviour of B-chromosomes in *Anthoxanthum aristatum*. As in rye, a slight reduction in pollen fertility and vigour of the plant is brought about by accessories, and as in rye (and in *Sorghum*) mitosis is belated in such pollen grains as have accessories. A non-disjunction occurs in the pollen mitosis, and this process shows the same appearance as in rye, the B-chromosomes of *Anthoxanthum* then being very similar to the large iso-fragment of rye. ÖSTERGREN finds, however, no evidence in his crossing results of a directed non-disjunction on the female side. The B-chromosomes of *Zea mays* must have a directed non-disjunction in the pollen, though this has not been demonstrated cytologically, but the genetical results also here show that a directed non-disjunction does not occur in the ovules (RANDOLPH, 1941).

I have investigated the development of the embryo-sac in a number of fragment plants of rye in the hope of finding a cytological demonstration of the directed non-disjunction that must occur on the female side. Spikelets of about 80 plants from the cultures of Professor MÜNTZING were fixed and imbedded in paraffin. As the spikelet only has two ovules, and as one rarely finds mitosis stages, it has been

necessary to prepare a very large number of slides. This has been possible thanks to a grant from »Statens naturvetenskapliga forskningsråd». The chromosome number of all investigated plants had been determined in root tips. Plants of »Östgöta Gråråg» having two or four fragments and of »Vasa II» with four or six fragments have been studied more closely. In plants with six fragments the first pollen mitosis was also studied. Most fixations were made in 1946, and when plant numbers without year number are given they are the field numbers from 1946.

## MEIOSIS.

The chromosome pairing could not be studied, because the youngest ovules observed already contained the second telophase stage or young macrospores. During the second division the spindle figure in the micropylar dyad cell is transverse or diagonal, in the chalazal dyad cell it is always longitudinal. Thus, the four macrospores resulting from the division form a so-called T-tetrad, the chalazal spore being the largest. Eliminated chromosomes were sometimes observed. Fig. 1 shows an unusually high elimination, this being in a plant having four accessories. Probably unpaired accessories behave as in the p. m. c's, in most cases dividing at the first division and often eliminated in the second division. Later, when three macrospores have degenerated, one may rarely observe a micronucleus or eliminated chromosome in the surviving chalazal macrospore. MÜNTZING has described the frequent occurrence of a »drop» in the young pollen grain. Such a drop was often observed in the chalazal macrospore: it has a homogeneous appearance and therefore does not resemble a micronucleus. Its constant size makes it probable that the drop is a transformed accessory, but in the macrospore it does not persist long; before the nucleus divides, it has disappeared.

The macrospore undergoes certain changes before the nucleus divides. One or two vacuoles may appear in its micropylar part, a more constant occurrence is a vacuole at its chalazal end. These vacuoles may arise before or after the division of the nucleus. Later, a vacuole always appears between the daughter nuclei. The spore increases in size before the first mitosis, but this increase is very variable. In plants having 18 or 20 chromosomes the spore and its nucleus are often very considerable before the division. In plants with a fewer number of chromosomes one may observe spores of nearly unchanged size but having two nuclei. Probably mitosis is delayed in macrospores having accessories (compare the behaviour of the pollen grains), and probably

this delay is more pronounced when the number of accessories is larger. But perhaps there is also a variation independent of the presence of accessories. It may be mentioned that one or two chalazal nucellus cells belonging to the same cellular row as the macrospores often have a nuclear structure and cytoplasm that are different from those of other nucellus cells. Such cells may resemble macrospores.

## THE FIRST DIVISION IN THE EMBRYO-SAC.

Prophase of the first mitosis has been observed many times and in more advanced stages it is possible to count the chromosomes. Normal metaphase stages were observed only twice. One is shown in Fig. 2. It is from plant 19—9 (17 chromosomes); the plate had 8 chromosomes, and its position was about midway between the micropylar and chalazal ends of the macrospore. In spite of a special staining (kindly performed by Dr. G. ÖSTERGREN) it was not possible to study the spindle more closely, the section being too thick. Anaphase was observed five times. Three of them were late stages and showed no lagging chromosomes; this might depend on the absence of fragment chromosomes in the macrospore, or possibly present fragments had already reached the pole. Two anaphases showed lagging chromosomes. Fig. 3 is from plant 19—9 mentioned above. Seven chromosomes are passing to each pole while on the spindle a standard fragment chromosome is lagging, having in contradistinction to the other chromosomes an undivided centromere. It is nearer the chalazal anaphase group. However, the chromosome is straight and thus shows no anaphase movement; probably it will later be included in the micropylar nucleus. The presence of a small vacuole had caused a slight displacement of the spindle. A further anaphase was from a plant with 16 chromosomes (8—12); here the fragment chromosome had the typical anaphasic appearance that was described and figured by MÜNTZING. In the latter plant there was also found a telophase where an elimination had occurred; two chromatids lay between the daughter nuclei: they were probably from the accessory that had been lagging and divided belatedly. This was however the only case of elimination observed. Fig. 6 shows a telophase from 24—12 (»Vasa II» with 18 chromosomes). The division is much delayed; judging from the size of the ovule and the embryo-sac one would expect at least the four-nucleate stage. This delay is probably the cause of the excentric position of the spindle near the micropylar end of the embryo-sac. This is exceptional: observed divisions, as well as the position of

the nuclei after the division, show that the first spindle is more often situated near the middle of the embryo-sac.

Fig. 5 is from a plant of »Vasa II» with 20 chromosomes (54—6). No regular plate has been formed, but the ten chromosomes have a tendency to be concentrated on two poles and most of them are divided. A peculiar behaviour of all the chromosomes also occurs in the pollen

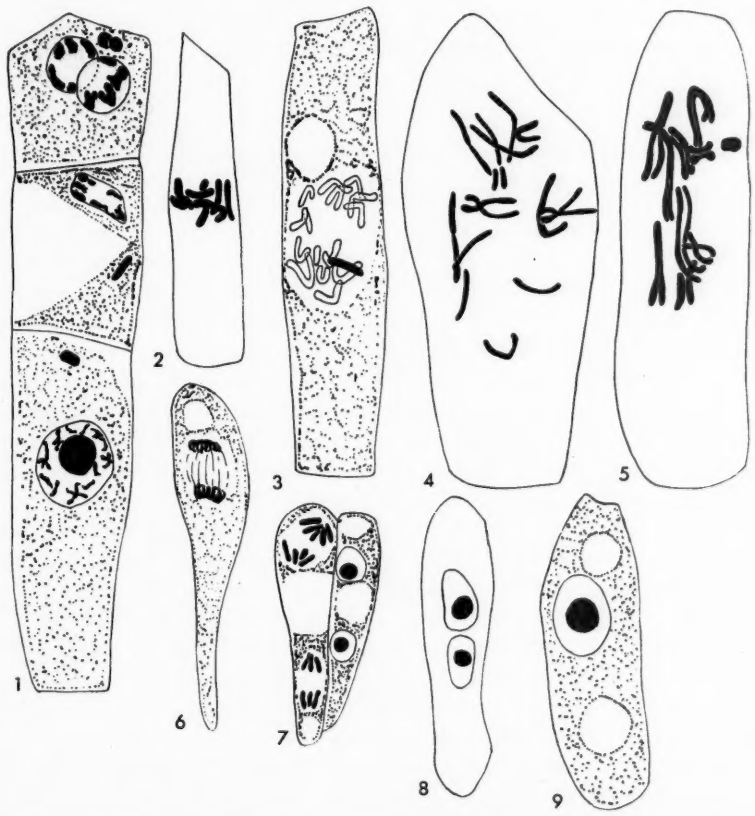



Fig. 1: macrospore tetrad from a rye plant with four fragments (× 1400). — 2: the first metaphase in the embryo-sac (× 600). — 3: anaphase with a lagging accessory (× 1400). — 4: disturbed first mitosis in the embryo-sac of a plant with four standard and two small iso-fragments (× 1400). — 5: the disturbed first mitosis in an embryo-sac with three fragments (× 1400). — 6: telophase of the first mitosis (× 600). — 7: two embryo-sacs in the ovule (× 600). — 8: two-nucleate embryo-sac from a plant with four fragments (× 600). — 9: one-nucleate embryo-sac from a plant with four fragments (× 600).

grains of plants with six accessories and will be described in a separate chapter of this paper. Fig. 4 is from plant 1947—255—10, an »Östgöta Gråråg» plant with 20 chromosomes, the accessories being four standard fragments and two small iso-fragments. Probably this is a somewhat abnormal anaphase. At the equator one observes two standard fragments having the typical anaphase appearance well known from the investigations of MÜNTZING. Small iso-fragments were not expected, because as a rule they are lost at meiosis, as MÜNTZING has found. The section with the figured chromosomes was the first in the slide and the complementary section could not be found.

## THE LATER DIVISIONS IN THE EMBRYO-SAC.

The observations on the first mitosis in the embryo-sac had shown that accessory chromosomes lag and may take the same appearance as at anaphase in the pollen grain. A directed non-disjunction could not be demonstrated; that it regularly occurs in the first mitosis is, however, clear from a study of the later divisions. Prometaphase of the second division is the stage most favourable to show this. Figs. 10 *a* and *b* are from an »Östgöta Gråråg» with 16 chromosomes. The prometaphase at the micropylar end shows nine chromosomes, while at the chalazal end only seven chromosomes could be observed. The satellite chromosome is conspicuous in both chromosome groups. Two standard fragments are seen to the right in the micropylar group. There can be no doubt that the nucleus of this macrospore had 8 chromosomes, and that the fragment chromosome has undergone non-disjunction, its two chromatids having passed towards the micropylar pole. Figs. 11 *a* and *b* show the two ends of an embryo-sac of plant 52—9, which was a »Vasa II» having 16 chromosomes. At the micropylar end are 10 chromosomes, two of them lying at some distance and clearly being fragment chromosomes. At the chalazal end there were no doubt 8 chromosomes. This makes 18 chromosomes, two more than expected. It is difficult to account for the high number of chromosomes. In »Vasa II» the pairing of fragments is good and the formation of a macrospore with both the fragments must be rather rare here. Be that as it may, as the functioning macrospore here had 9 chromosomes a premeiotic or meiotic non-disjunction (of perhaps an A-chromosome) must have occurred. Figs. 12 *a*, *b* and *c* show prometaphase of the last division in the embryo-sac from a plant (29—6) having 18 chromosomes, two of them standard fragments and two being large iso-frag-

ments. Each of the chalazal chromosome groups had 7 chromosomes. One of the micropylar divisions clearly showed 11 chromosomes, the other had been divided by the microtome knife, but no doubt had the same chromosomes. The macrospore had possessed nine chromosomes, and at the first mitosis the standard fragment as well as the large iso-

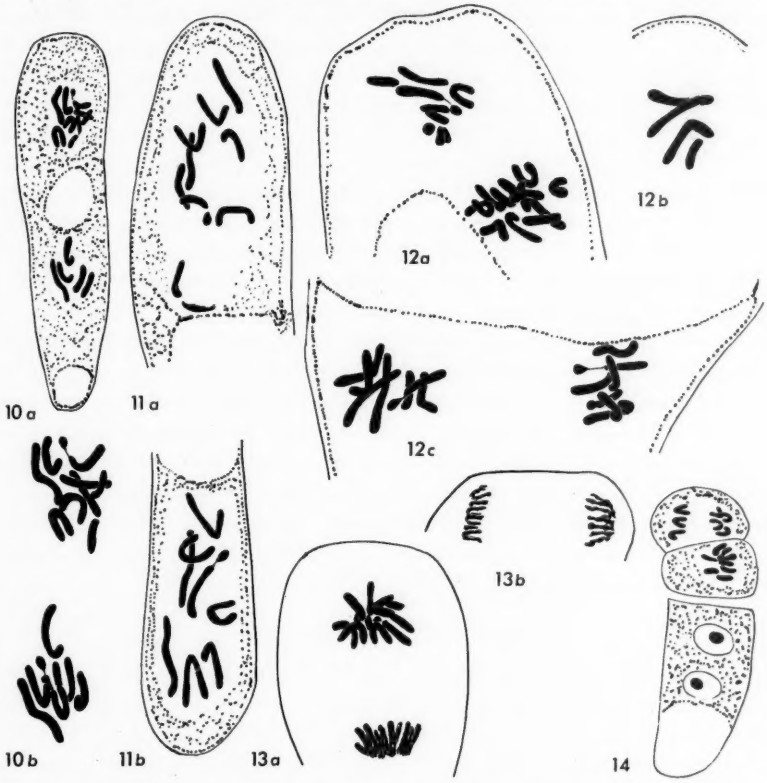



Fig. 10 *a*: prometaphase of the second division from a plant with two fragments (× 600). — 10 *b*: (× 1400). — 11 *a*: the micropylar, 11 *b*: the chalazal, prometaphase from a plant with two fragments. — 12: prometaphase of the third division from a plant with four fragments. *a* and *b* the micropylar, *c* the chalazal, end of the embryo-sac (× 1400). — 13 *a* and *b*: late anaphase of the third divisions at the micropylar end from a plant with four fragments (× 1400). — 14: antipodals from a fragment plant (× 600).

fragment had undergone non-disjunction directed towards the micropylar pole.

There were other cases where the number of chromosomes could be determined at each end of the two-nucleate or four-nucleate embryo-sac. Prophase stages are the least rare but in most cases they do not allow a sure determination of the number of chromosomes; prometaphase is a favourable stage, meta- or ana-phase is less favourable. In six cases there were 7 chromosomes at each end, all being found in plants of »Östgöta Gråråg» with 15 or 16 chromosomes; the accessory chromosomes had here been eliminated at meiosis. Twelve cases in all showed a higher number of chromosomes at the micropylar end of the embryo-sac. In seven embryo-sacs the chromosomes could be counted in antipodal cells (Fig. 14). Their number was always seven. Most of these embryo-sacs probably had fragment chromosomes, and it must be a rule that fragment chromosomes are present only in the micropylar nuclei of the embryo-sac, owing to non-disjunction at the first mitosis. Fig. 11 discussed above shows the only *certain* case where more than 7 chromosomes were observed at the chalazal end.

Ana- and telo-phase stages of the second and the third division in the embryo-sac have several times been observed. They were quite regular, no lagging or elimination of chromosomes could be detected in such divisions as must have had fragment chromosomes. Figs. 13 *a* and *b* are from a plant with 18 chromosomes and show the third division at the micropylar end; *a* is the mitosis resulting in egg nucleus and polar nucleus, *b* the mitosis giving the synergid nuclei. Thus it seems that the rye accessories behave as normal chromosomes in the second and third division and that eliminated fragments do not as a rule occur.

A few times there were two embryo-sacs in the nucellus. Fig. 7 is from an 18-chromosome plant. It shows one two-nucleate embryo-sac and one with anaphase of the second division. The micropylar anaphase had clearly more chromosomes than the chalazal, but the chromosomes could not be counted exactly. It is not possible to decide whether the embryo-sacs come from the same macrospore tetrad, or whether they belong to two different tetrads, the ovule having contained two embryo-sac mother cells. However, in another case (also from an 18-chromosome plant) two two-nucleate embryo-sacs lay in a row, showing that they belonged to one macrospore tetrad.

The newly organized embryo-sac is rather small but later increases in size. The antipodals divide and this cell group soon consists of three or four storeys (cf. Fig. 14): (1) a long chalazal antipodal that sometimes had undergone transverse division, (2) two or four rather large cells.

(3) four or more cells that are relatively broad. The polar nuclei soon enter into contact but do not fuse. One might expect that the polar nuclei would often be of different size, in most embryo-sacs from plants with four fragment chromosomes, one polar nucleus must have seven, the other eleven, chromosomes. However, a clear size difference could not, as a rule, be observed and the nucleolus in each of the nuclei had about the same size. The number of organized embryo-sacs observed was small however.

Any influence accessories may have on size of nucleus and nucleolus might be shown by a comparison of the micropylar and chalazal nuclei in the developing embryo-sac. However, I am inclined to believe that this is not the case, for the nuclei undergo divisions and are in a dynamic condition, and the chalazal nuclei are in another and more advanced stage of division than the micropylar. The latter difference is not a result of a retardation of the micropylar divisions caused by the accessories; such a difference is also to be found in embryo-sacs with 7 + 7 chromosomes and is a regular feature of the angiosperm embryo-sac that has been ascribed to »mitotic hormones» entering the embryo-sac from the chalaza and spreading to the micropylar end. In view of these facts it seems unsafe to draw conclusions from the appearance of the nuclei in the two- to eight-nucleate embryo-sacs. Many times it was observed that the micropylar nucleus or nuclei were larger and had a larger nucleolus (Fig. 8), but a size difference was also observed in a plant lacking fragment chromosomes.

## ANOMALOUS EMBRYO-SACS.

Disturbances may occur in the development in the ovules. Sometimes no embryo-sac is formed, because all four macrospores degenerate. This was also observed in normal plants, where it cannot be caused by the presence of accessories. Certain other disturbances seem preferentially to occur in plants with more accessories than two. Only some of them will be mentioned: unusual distribution of the vacuoles in the embryo-sac, great delay of the first division (a plant with four large iso-fragments once had an embryo-sac showing prophase of the first mitosis with the same size as at the eight-nucleate stage), abnormal development of the somatic tissue of the ovule (in a plant with three large iso-fragments the integuments of the ovule were rather often reduced).

More interesting is the occurring of embryo-sacs with a more in-

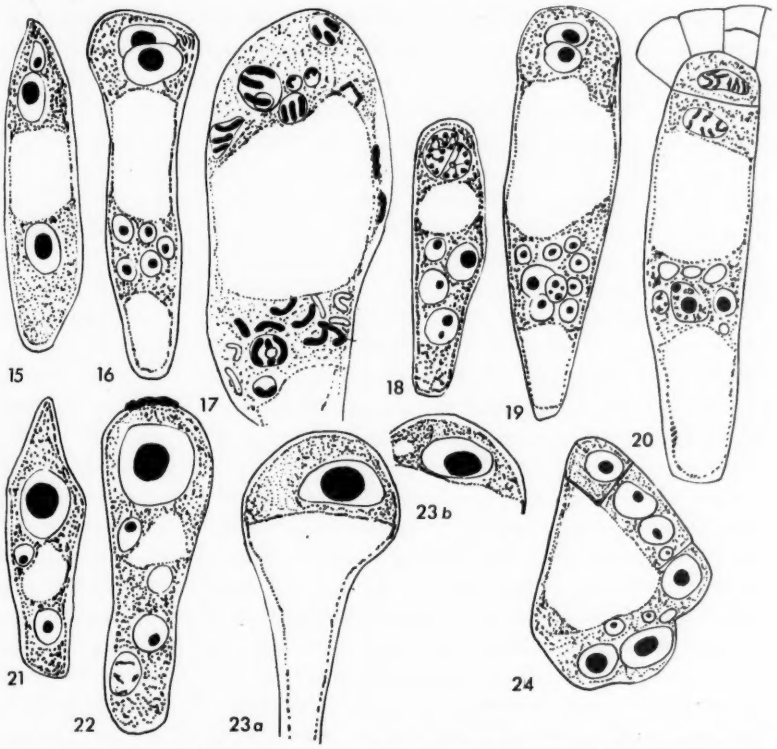
determinate number of nuclei showing different size. Such embryo-sacs were rather common in three 20-chromosome »Vasa II» plants fixed in 1946 (54—2, 54—6 and 55—6), they were also though less often found in 1947—262—16 (20 chromosomes) and in 1947—255—10 (a plant having four standard fragments and two small iso-fragments). Thus, all 20-chromosome plants investigated have shown this peculiar disturbance. But it may also occur in plants with 18 chromosomes, for instance not infrequently in 1947—256—10, 1947—262—12, and some of the plants fixed in 1946. In other 18-chromosome plants this disturbance was not observed, but perhaps it would have been found if more material had been investigated. However, the special disturbances were never observed and are probably very rare in 16-chromosome plants.

The principal cause of the irregular nuclei in the embryo-sacs with a higher number of fragment chromosomes is no doubt disturbance of the first mitosis. Two such cases have been figured above, and the formation of supernumerary nuclei at the first pollen mitosis is frequent in 20- and 18-chromosome plants. As division stages only very rarely can be observed, a closer cytological investigation could not be made.

Two embryo-sacs from 1947—256—10 are shown in Figs. 15 and 16. Fig. 15 shows a two-nucleate stage with a small supernumerary nucleus at the micropylar end, presumably some chromosomes in the larger anaphase group of the first mitosis had been at a certain distance and formed a separate nucleus. In Fig. 16 there are two large micropylar nuclei and five small chalazal nuclei. Probably the second division had been disturbed at the chalazal end, resulting in the formation of several nuclei of varying size. Other cases of an abnormal number of nuclei were found; one embryo-sac had, for instance, four nuclei at each end, but the nuclei in each group showed size differences. 1947—262—12 showed similar embryo-sacs; here an embryo-sac was found with an egg apparatus at the micropylar end but no cell organization at the chalazal end, the antipodal apparatus being represented by a group of free nuclei of different size. Normal embryo-sacs are of course often observed in these two plants.

In all 20-chromosome plants investigated embryo-sacs with supernumerary nuclei seem to be frequent. Of 54—2 only a scanty material could be observed because the chalazal macrospore had very often degenerated. This plant also had a high pollen sterility, only about 40 % of the pollen grains being good. Plant 55—6 more often developed an embryo-sac, although this was frequently abnormal. Fig. 19 shows an embryo-sac with two large micropylar nuclei and nine chalazal nuclei

of different size. This embryo-sac is rather large. A smaller one with two larger micropylar nuclei lying close together with a surface of contact and four chalazal nuclei at the other end is shown in Fig. 18. Another anomaly was one nucleus at the micropylar end, two at the




Figs. 15—24. Abnormal embryo-sacs. — 15 and 16: from a plant with four fragments (× 600). — 17—24: from plants with six fragments (× 600; 17, × 700).

chalazal. Supernumerary nuclei may also be found at the micropylar end of the embryo-sac, but seem to occur less often here.

From plant 54—6 division stages were obtained. An abnormal first mitosis has already been figured and described on p. 39. Fig. 17 shows the formation of many small nuclei at both ends of the embryo-sac. A neighbouring section was lost and therefore not all the chromosomes could be detected. At the chalazal end there is a small nucleus formed of three chromosomes, a micronucleus formed of one chromo-

some, and eleven free chromosomes that will probably form some nuclei later. At the micropylar end there were four nuclei, and a few micronuclei, but still more nuclei must have been present in the lost section. This case throws further light on the origin of the supernumerary nuclei that are so frequent, particularly at the chalazal end of the embryo-sac. The embryo-sac figured can hardly be in the first mitosis stage, probably it shows an abnormal anaphase-telophase of the second division (there is also the possibility that it may be the third division). It seems clear that the chromosomes have undergone anaphasic movements, though no polar groups have been formed, perhaps owing to defective spindles. Another division observed had a quite different aspect. One sometimes observes a very large embryo-sac with an unusually large nucleus (cf. Fig. 9). It was the division of such a nucleus that was observed. The section containing this division was too thick, and notwithstanding many trials I did not succeed in obtaining a microscopic picture possible to figure exactly. The number of chromosomes was large and I got the impression that a nucleus with the unreduced chromosome number was dividing. Perhaps the first mitosis had resulted in one restitution nucleus instead of the normal micropylar and chalazal nuclei, and it was the division of such a nucleus that had been fixed.

In this plant supernumerary nuclei were very often observed. A further disturbance was a premature cell formation at the micropylar end. Fig. 20 shows two large nuclei in this part of the embryo-sac. They are separated into different cells; at the chalazal end there were at least seven nuclei. Figs. 23 *a* and *b* show a large embryo-sac having two very large micropylar nuclei, probably daughter nuclei of a restitution nucleus, while at the narrow chalazal end no nucleus could be detected. A few older, organized embryo-sacs were found. The antipodal apparatus was lacking or consisted of only one or two cells. An aberrant embryo-sac with more than three cells at the micropylar end and no polar nuclei and antipodals was observed. However, only few spikelets having such old stages had been fixed.

In the 20-chromosome plants one may also observe quite regular two- and four-nucleate embryo-sacs. From plant 1947—262—16 older stages could be studied. The disturbances did not seem to be as frequent in this plant; an embryo-sac with one large and two small nuclei is shown in Fig. 21, and another with one very large and four small nuclei in Fig. 22. The organized embryo-sac is broad and may have a quite regular appearance, with a normal antipodal group. Abnormal embryo-sacs do, however, occur and one of them is shown in Fig. 24. The

cellular organization is incomplete, there is only one cell at the micropylar end, the nuclear differences are very great.

Thus, in plants with six rye accessories not only the first but also the following divisions in the embryo-sac are often disturbed. These disturbances may no doubt result in a certain sterility owing to abnormal embryo-sacs; however, normal embryo-sacs also occur and 20-chromosome plants have given good kernels though in a rather low percentage (cf. MÜNTZING).

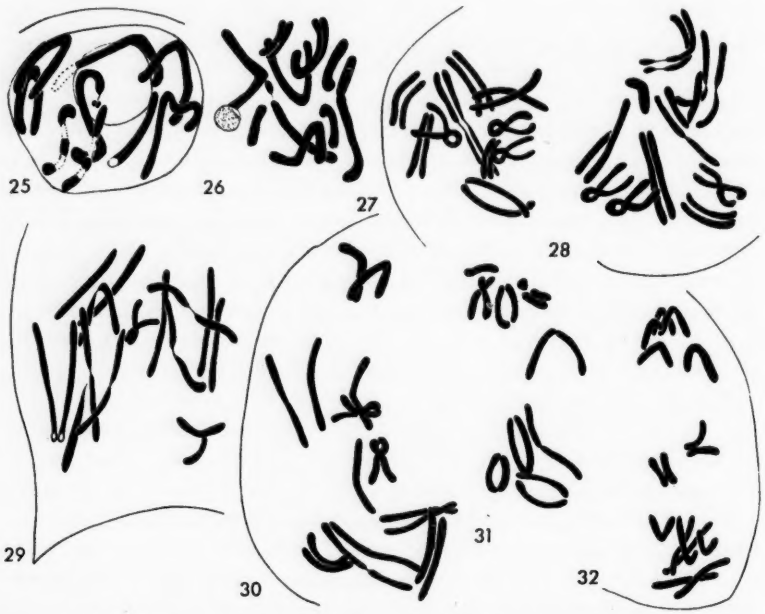
## POLLEN MITOSIS IN PLANTS WITH SIX FRAGMENTS.

Only one first mitosis could be found in the embryo-sac of 20-chromosome plants, and the observed division was very irregular. However, the high frequency of abnormal embryo-sacs in all such plants indicated that disturbance of the first division must be rather frequent. As all spikelets that could show this stage in the ovules had been investigated, I turned my attention to the anthers in the slides. Notwithstanding the fact that the slides had been made in routine manner, good divisions could be studied from the three »Vasa II» plants mentioned above. They all showed a very peculiar disturbance of the pollen mitosis. A similar disturbance also occurred in 1947—262—16, though here the divisions were more indistinct.

The greatest number of divisions were observed in plant 54—2. The most common chromosome number in the grains seemed to be 10. This was not unexpected, because »Vasa II» fragments show good pairing (meiosis has, however, not yet been studied in plants with six fragments). As will be shown later in a plant with four fragments, the disturbances are more pronounced when the pollen grain has a higher chromosome number. Before division the pollen nucleus has the usual position near the wall. A prophase is shown in Fig. 25. The centromeres of the A-chromosomes in this nucleus are directed towards the wall of the pollen cell; they have not changed their position from the preceding telophase. In the B's there are large destained segments. As such segments have been observed in only a few nuclei, it seems unsafe to draw conclusions concerning the nucleic acid content of the B-chromosomes compared with that of the A-chromosomes.

When the nuclear membrane has disappeared, the irregularities set in. A spindle seems, as a rule, to be formed but in most cells no regular metaphase plate. The chromosomes may have very different positions. They may be strewn along the whole length of the spindle, most of them

lying in its longitudinal direction. In most cells the spindle is parallel to the short end of the cell, though it may also be perpendicular or have intermediate positions. The chromosomes may form a group, but then a few of them often lie at a distance from the majority. A variable number of chromosomes may form a plate, and sometimes most or all centromeres are in a plate or show a tendency to form one. Usually in the latter case all the centromeres do not lie in the same plane




Figs. 25—32. The first pollen mitosis from plants with six fragments (× 2200). — 25: prophase. — 26: metaphase in side view. — 27—32: compare description in the text.

(Fig. 26) and often seem to have a faulty orientation in relation to the poles of the spindle, longitudinal instead of transverse (cf. Fig. 29).

The variable positions of the chromosomes in the cell are shown by the figures that illustrate the following phase: in the disturbed divisions the centromeres soon split, and pairs of chromatids lie as in a c-mitosis. Fig. 27 shows a case from 55—6. An interesting feature is the form of the B-chromosomes. They all have the bivalent appearance discovered by MÜNTZING. It seems as though this appearance is caused merely by

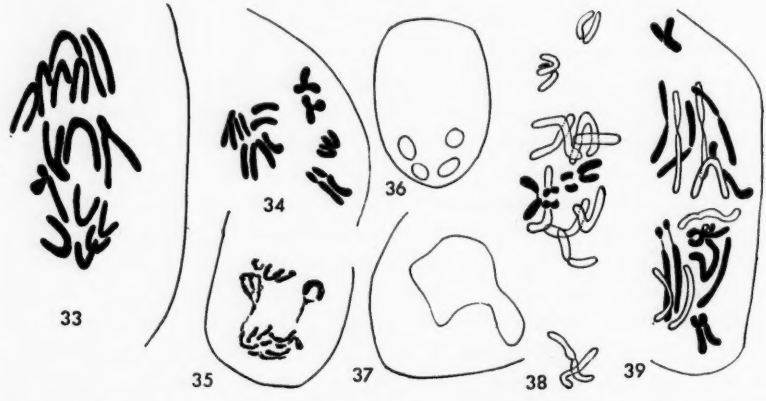
the division of the centromere (and of course the non-division or stickiness of the special segment), and not from the influence of pulling or pushing forces on the daughter centromeres. The short arms are all directed towards the centre of the chromosome group. Fig. 28 is from 54—2. In this cell the chromosomes were all lying in about the same plane but the centromeres were not orientated as in a regular plate. The fragments have here the short arm and thus the centromere directed peripherally (the fragment at ten o'clock was rather indistinct, its centromere had perhaps not divided). In one of the A-chromatid pairs at the centre sisters from one of the arms are widely separated. Fig. 29 shows a somewhat later stage. Here the chromatid pairs are, as it seems, in the longitudinal direction of the spindle. Unfortunately one chromatid, one arm of a chromatid and probably one B were missing. The figure shows that sister chromatids may move a short distance apart, and at least in one pair there seems to be a true anaphase separation through traction fibres. The chromosomes here are still long and thin.

Thus, there is a certain displacement of chromatids on the spindle that is probably caused by streamings in the spindle and changes in the size and form of the spindle before the telophase sets in. It seemed as though sister chromatids nearer the middle or in broader regions of the spindle were moved further apart than chromatids near the ends of the spindle. Such a case is shown in Fig. 30. Here only two B-chromosomes were detected, one of them having a normal orientation as if lagging in an equatorial plate, the other having no such orientation. The position of the latter cannot be due to true anaphase movement; had this been the case, the short arm would have been directed to the lower pole. One chromatid pair is at the upper, four at the lower, end of the spindle. Two sister chromatids in the centre have moved apart in a longitudinal direction without showing evidence of being pulled by traction fibres. Fig. 31 shows polar groups of chromosomes and a true anaphase separation of two sister chromatids; all the chromosomes observed were not figured in this case.

The number of pairs where an anaphase separation through traction fibres occurs is varying and no doubt largely depends on the number of chromosomes forming a metaphase plate. The division in Fig. 32 has two chromatid pairs at the lower (generative) pole, while in the other A pairs normal anaphase has occurred. In this cell only two B-chromosomes seemed to be present, the one lagging, the other showing non-disjunction towards the generative pole. Normal anaphase separation

in five pairs has occurred in Fig. 33. Here four elements lag, three of them are B-chromosomes of unusual size, one is an unsplit A-chromosome (or an A-chromatid of unusual diameter).

When the telophase changes set in, the chromosomes may show very different positions. There may be two polar groups and two nuclei are formed. Chromosomes lying between the groups may give rise to bridges. As two sister chromatids are very often included in the same nucleus, deficient nuclei must be frequent, and often three or four nuclei are formed. Fig. 34 shows a cell where probably four nuclei will result, and in Fig. 35 there are bridges between the nuclei. No doubt a




Figs. 33—39: The first pollen mitosis. 33—37 from a plant with six fragments, 38—39 from a plant with four fragments. — 33: anaphase (× 2200). — 34: telophase (× 1400). — 35: late telophase (× 1400). — 36: pollen grain with four nuclei (× 600). — 37: irregular restitution nucleus (× 1400). — 38: disturbed anaphase, two fragments (× 2200). — 39: strongly disturbed mitosis, three fragments (× 2200).

restitution nucleus is sometimes formed (cf. Fig. 37), but it was impossible to form an opinion of the frequency of such nuclei.

An investigation of later stages has not been made. A generative cell of normal appearance had sometimes been formed. In such cases the pollen mitosis had probably been rather regular, and in most cases pollen grains with such a cell probably had only one or two fragments. When the pollen mitosis is disturbed, non-functional pollen grains must result. It is difficult to see how a generative nucleus could be formed when the pollen mitosis had given rise to a restitution nucleus, or to supernumerary nuclei. Grains with three or more free nuclei and not divided into a generative and a vegetative cell were observed. Grains

with a degenerating generative cell were also seen. Plants with six fragments must have a rather high pollen sterility, but functionless grains that cannot germinate may often have a morphologically rather normal appearance. Plant 54—2 had had about 40 %, 54—6 about 60 % and 55—6 about 80 % seemingly good pollen (to the sterility of plant 54—2, however, factors other than disturbed pollen mitosis probably contribute; cf. p. 44). As the first mitosis occurs when the pollen grains have attained a certain size, the disturbances cannot have such great morphological effects as an irregular meiosis.

## POLLEN MITOSIS IN A PLANT WITH FOUR FRAGMENTS.

The observations on the pollen mitosis have thrown light on the origin of the frequent irregularities in the embryo-sacs of plants with six fragments. A disturbed first mitosis may cause the formation of supernumerary nuclei or of a restitution nucleus. Now, supernumerary nuclei also were observed in embryo-sacs of plants with four fragments, though less frequently. It seemed probable that they had a similar origin here. The only first mitosis observed that showed some irregularity was from a plant having not only four standard fragments but also two small iso-fragments. Fortunately some slides from an »Östgöta Gråråg» plant with four standard fragments (11—4) had sufficiently good pollen divisions to allow of cytological observations. I have not, however, studied the pollen mitosis very closely.

The pollen grains had one to three B-chromosomes (other numbers perhaps also occur). The most frequent chromosome number seemed to be nine, but grains with eight chromosomes were also numerous, while grains with 10 chromosomes are probably less frequent. A number of pollen grains showed similar disturbances as those described from plants with six B-chromosomes. A metaphase plate was not formed, but unsplit chromosomes or, much more often, pairs of chromatids lay in different positions on the spindle. Such a disturbance is shown in Fig. 39. Here one B-chromosome is at each pole of the spindle, while a third B is slightly below the equator. Six pairs of sister chromatids are in the longitudinal direction of the spindle. They form two groups, in each of which the unsplit centromeres seem to have been in the same plane. One A-chromatid pair has a transverse position with the upper chromatid as a V, indicating the influence of a polar traction fibre. Pollen grains with a restitution nucleus or supernumerary nuclei were also observed.

The investigation of the plants with 20 chromosomes had given some evidence that the degree of disturbance of the mitosis was correlated to the number of B's present in the pollen cell. This was much clearer here. The strongly disturbed divisions had ten chromosomes.

Most pollen cells exhibit a regular or nearly regular metaphase plate; where the number of chromosomes could be determined it was eight or nine. In other cases the metaphase plate was less regular, there was evidence of non-congression, and one or two chromosomes could be at the poles of the spindle. Cells with less regular plates of this kind had most frequently nine chromosomes. Regular anaphase stages with one or two B-chromosomes lagging were often seen. It is difficult to count the chromosomes in a cell with anaphase; however, when one B is lagging the cell must in most cases have eight, when two B's are lagging, nine chromosomes. The observations show that, as a rule, when the pollen has eight chromosomes a regular metaphase plate is formed and anaphase proceeds in the manner described by MÜNTZING from »Östgöta Gråråg» plants with 16 chromosomes. In pollen cells with nine chromosomes metaphase and anaphase are often regular, though of course two B-chromosomes lag (they were also observed on the way to the generative pole). But in cells with nine chromosomes the anaphase may also be rather disturbed. A moderately disturbed division of this nature is shown in Fig. 38. Here one A-chromatid pair is at the lower pole and one at the upper, while one pair is nearer the equator. The chromatids of such A-chromosomes as had formed a metaphase plate have begun their movements to the poles. The B-chromosomes lag at the equator and show a peculiar appearance, having a rather large colourless section in the long arm. The position of the centromere could be observed in one of the B's. This »quadripartite» form of the two B-chromosomes was observed in several pollen cells. It seems as though it was always the same region of the chromosome which was destained, and this must include the special segment of MÜNTZING. This peculiar appearance of the B-chromosomes was only observed in pollen cells with nine chromosomes and a bipolar spindle with anaphase groups.

In two rows of a slide I made some counts. Of 24 very regular plates, 12 had eight and 12 nine chromosomes. Of 25 nearly regular plates, 4 had eight and 21 nine chromosomes. Of more irregular metaphase stages, 5 had nine and 2 ten chromosomes. Nineteen cells had the strongly disturbed division so frequent in the 20-chromosome plants, they all had ten chromosomes. The high number of cells with 10 chro-

mosomes depends on the fact that they include cells in which the chromatids were split, and such cells were in the majority. Most of the cells showing a regular division (meta- to late ana-phase) had unsplit chromosomes, while the reverse was true of the cells with a strongly disturbed division. This difference may be explained if in the latter case the development after the splitting of the centromere is very slow. An earlier division of the centromere may also contribute to the difference, though this seems rather improbable. It may be mentioned that a diploid pollen cell was observed. The chromosomes had formed a regular metaphase plate, but the position of the plate in the cell was somewhat unusual: it was quite close to the wall, the generative pole of the spindle being very short and broad. Unfortunately the number of B-chromosomes could not be determined here, perhaps the influence of the B-chromosomes on the pollen mitosis is less when 14 instead of 7 A-chromosomes are present (compare the regularity of the mitosis in the soma). A much larger number of divisions than those just accounted for were studied in the slides from 11—4. The observations confirm that when one B-chromosome is present the division is normal with directed non-disjunction of the B-chromosome; when two B-chromosomes are present the division in most cases is regular, but may sometimes be more or less disturbed with chromatid pairs near the poles, while the presence of three B-chromosomes as a rule destroys the normal mitotic mechanism. Only one plant has been investigated, but similar conditions probably exist in other 18-chromosome plants.

The supernumerary nuclei in certain embryo-sacs of 18-chromosome plants thus seem to be caused by a disturbed first mitosis. Probably in many cases these anomalous embryo-sacs had three fragments. Normal embryo-sacs where eleven chromosomes could be counted in micropylar divisions and seven in chalazal divisions were several times observed. This shows that a macrospore nucleus with nine chromosomes may undergo a normal anaphase with a directed non-disjunction of the two B-chromosomes. However, as in pollen grains with two B-chromosomes, the division is probably sometimes disturbed. Thus, some of the anomalous embryo-sacs may have had only two B-chromosomes. The relative high frequency of anomalies in two of the 18-chromosome plants, compared with a low frequency in other plants, indicates a certain lability of the first mitosis in macrospores with two fragments; external influences may alter its course rather easily. Normal embryo-sacs in 20-chromosome plants in most cases probably had only one or two B-chromosomes.

## CONCLUSIONS.

It has been shown that at the first mitosis in the embryo-sac the standard fragment of »Östgöta Gråråg» and of »Vasa II», as well as the larger iso-fragment of »Östgöta Gråråg», as a rule undergoes non-disjunction directed to the micropylar pole. The micropylar nuclei including the egg nucleus thus get an even number of these chromosomes, whereas they are absent from the nuclei at the chalazal end of the embryo-sac. The fact that those egg nuclei which function as a rule have an even number of fragments has been shown by the crossing experiments of MÜNTZING, and this is brought about in the same manner as when the sperms get an even number. Exceptions from the directed non-disjunction seem to be rare. Once, an elimination of the fragment had occurred; it had divided at the equator but the chromatids had failed to reach the poles before telophase. During the second and third divisions in the embryo-sac the fragments behave normally and non-disjunction or elimination does not occur. It may be pointed out that the mitosis which results in egg nucleus and upper polar nucleus is also normal. As the telophase group that forms the egg nucleus is always directed towards the end, and the polar nucleus group is turned towards the centre of the embryo-sac, one could perhaps suspect an asymmetry like that occurring when the generative cell is formed in the pollen grain. However, the formation of cell membranes does not occur in connection with mitosis but later, and an asymmetry of this spindle has never been described.

Only a few times was the anaphase of the first mitosis observed in the embryo-sac. The peculiar bivalent form of lagging standard fragments described by MÜNTZING has, however, been observed. An explanation of the second stage in the peculiar behaviour of the B-chromosomes in the pollen mitosis — their preference for the generative pole — could, as ÖSTERGREN (1947) points out, be found in the well-known asymmetry of this division. It is manifested in an asymmetrical position of the spindle with the generative end always in contact with the wall, in an asymmetry of the metaphase spindle, the generative pole being blunt, in a difference of the anaphase movements between the generative and the vegetative chromosome group, and in an early difference of the structure of the two daughter nuclei (cf. BRUMFIELD, 1941). Lagging and directed non-disjunction of B-chromosomes have been found not only in rye and *Anthoxanthum* but also in pollen grains of *Sorghum purpureo-sericeum* (DARLINGTON and THOMAS, 1941). The first mitosis

is normal in *Sorghum*, B-chromosomes, if present, behaving as A-chromosomes. However, in such pollen cells as have B-chromosomes the vegetative nucleus divides and a second generative nucleus is formed (»polymitosis»). In this extra division the B-chromosomes lag, they seem to take a similar appearance as the large iso-fragment in rye, and pass to the generative pole. It is the sperms from such secondary generative cells with an even number of B's that function.

An asymmetry of the first mitosis in the embryo-sac has never been shown, only the position of the spindle may be somewhat asymmetric, often slightly nearer the micropylar end. In the rye plants the spindle seems to be in the middle or more or less near the micropylar end, and it is difficult to understand why lagging elements should preferentially be included in the micropylar nucleus. Be that as it may, it is a striking fact that the fragments behave normally in all mitotic divisions with two exceptions: the first mitosis in the microspore or, as the case may be, the macrospore. These two divisions are homologous in the phylogenetical sense, they initiate the germination of the spores and the formation of a reduced male and female gametophyte. The first division shows that there is a polarization in the microspore, the difference between a generative and a vegetative pole being evident. A similar polarization is shown in the macrospore, though it finds its morphological manifestation only after the third division. Then the cellular organization sets in and the generative character of the micropylar end of the embryo-sac is shown. However, it seems very probable that the macrospore is polarized earlier, though it cannot manifest itself before a cellular differentiation has occurred. One may be inclined to conclude from the behaviour of the accessory rye chromosomes in the first mitosis that it is a proof of such an early polarization, and hope that it may be possible to show an asymmetry of this spindle.

It is very probable that the preference of the micropylar pole depends on an asymmetry in the organization of the first spindle. But perhaps this asymmetry is a rather special one caused by the fragment chromosomes of the nucleus and not manifest when such chromosomes are absent. The influence of B-chromosomes on the divisions in the pollen grain is shown in *Sorghum*, where the vegetative nucleus only divides in such pollen grains as contain B-chromosomes. And this new mitosis introduces the directed non-disjunction of the B-chromosomes. In rye, however, the influence of fragment chromosomes is still clearer, the presence of three such chromosomes causing considerable disturbances of the first mitosis in micro- and macro-spores.

ÖSTERGREN has obtained evidence that the forces causing the movements of the centromeres to a regular metaphase plate are attractions of the centromeres to the poles; the attraction forces are »traction fibres» that are set perpendicular to the centromere. Because the centromere is functionally divided already at metaphase it is suspended halfway between the poles of the spindle (ÖSTERGREN, 1945, and other papers). When a haploid rye microspore or macrospore has three fragment chromosomes, the normal centromere-spindle mechanism does not function. The cell has a spindle but a normal metaphase plate is not formed, though a variable number of centromeres may approach the plate conditions. However, in most cells there is no evidence of a plate, the chromosomes are mostly placed in the longitudinal direction of the spindle. The spindle is accordingly »weak» and may show signs of multipolarity. The centromeres divide normally and, as it seems, their division is not belated. The influence of traction fibres during anaphase may be shown in a variable number of chromatids, but very often sister chromatids are included in the same nucleus. Displacements of chromatids through streamings in the spindle seem to occur, changes in the form and size of the spindle, especially in »late stages», contribute to the formation of separate chromosome groups and thus separate nuclei.

Doubtless the disturbed division is very slow when compared with a normal bipolar anaphase. The pairs of chromatids lying around give the disturbed spore mitosis the resemblance of a c-mitosis. However, a comparison with the well-known description of a typical c-mitosis by LEVAN (1938) shows certain differences. Thus, it seems that the division of the centromeres is not belated (cf. p. 53). Another difference is that in most cases more than one nucleus results, the formation of a restitution nucleus seems to be more rare. In this respect the division resembles the »distributed c-mitosis» observed in *Allium* roots after treatment with certain types of vitamin K (NYBOM and KNUTSSON, 1947). It may further be stressed that the disturbed rye mitosis as a rule has a spindle.

LEVAN (1940) found that acenaphthene has a less strong effect than colchicine on the mitosis in *Allium* roots. In certain cells only one or two chromosomes behaved in the anomalous manner and in other more disturbed divisions »instead of being juxtaposed parallelly, as after colchicine treatment, the chromatids now move away from each other with the centromeres in front of them». ÖSTERGREN (1944) summarizes the results of the application of different concentrations of certain sub-

stances on *Allium* roots: »If a substance which is able to give full c-mitosis» (complete inactivation of the spindle) »is applied in more dilute solutions *a partial c-mitosis* may be obtained. The spindle is not completely inactivated, but it is unable to accomplish a normal chromosome distribution, the result is multipolar anaphases. When an even more dilute solution is applied, there occurs an intermixture of normal mitosis among the disturbed ones, and at still lower concentrations all mitoses are normal». In the literature on external influence of mitosis one finds many descriptions of divisions intermediate between »full c-mitosis» and normal mitosis.

No doubt there is a parallelism between the influence of different concentrations of certain chemicals on the root mitosis in *Allium* and that of a different number of accessory rye chromosomes on the first mitosis in the pollen cell and the embryo-sac. When one accessory is present mitosis is regular though the accessory behaves in its peculiar manner. Two accessories have an influence intermediate between that of one and of three such chromosomes. Mitosis is often normal with regular anaphase, but may be more or less disturbed. Thus the influence of accessory chromosomes on mitosis increases with their number in the haploid spore. In no case was a division with four observed. Clearly the presence of only one accessory must influence the spindle and perhaps cause the rather special asymmetry that results in the directed non-disjunction. Two B-chromosomes put a stress on the spore mitosis, and with three it breaks down. It would be of interest if the influence of B-chromosomes in diploid spores could be studied. I have only observed one such diploid division; it showed a metaphase plate, but the number of B-chromosomes could unfortunately not be counted. Perhaps a diploid number of A-chromosomes may neutralize the influence of four B-chromosomes.

There are strong indications that not only the first but also the following divisions may be disturbed in such embryo-sacs as have three B-chromosomes. Judging from the appearance of the abnormal embryo-sacs, the chalazal divisions are more often disturbed than the micropylar. This seems strange, because one would expect that in most cases there are more chromosomes and B-chromosomes at the micropylar end. Perhaps the disturbed second divisions are not due to the influence of B-chromosomes, but are a result of deficient chromosome equipment of the dividing nuclei, the first mitosis being disturbed. It may be pointed out that normally a central vacuole appears in the embryo-sac after the first division. As the strongly disturbed mitosis

is very slow, it probably occurs that vacuole formation has an influence on the chromosome distribution.

In the strongly disturbed divisions with no plate the fragment chromosomes may have the bivalent appearance described by MÜNTZING. Here, no pulling forces from traction fibres could have contributed to the bivalent form, which in this case is caused only by the division of the centromere (and by the non-division or stickiness of the special segment). ÖSTERGREN (1943) has shown that mutual elastic pressure between chromatids and chromosomes at their point of contact gives a simple explanation of the repulsions observed in c-pairs and bivalents. In the standard fragment the special segment offers such a contact point. There is thus, according to ÖSTERGREN, no need to assume special electrostatic repulsion forces between the daughter centromeres. Near the base of the long arm a rather large destained region that must include the special segment may be observed. The destained region was only observed in 11—4, the only plant with 18 chromosomes whose pollen division was studied, and only in pollen cells with two fragments and a bipolar anaphase spindle. It indicates a different nucleic acid »charge» in the special segment.

There is a number of papers on pollen mitosis disturbed through external agents. In this connection the paper of KOLLER (1943) on the influence of X-rays may be cited. A complete absence of a spindle or a delay in spindle formation may result, causing a disturbed anaphase and supernumerary nuclei. X-rays, however, have also and more often caused structural changes of the chromosomes, which makes the comparison with the influence of accessory chromosomes difficult.

In maize, interchanges between A- and B-chromosomes have been obtained by X-raying B-pollen. By genetical methods ROMAN (1947) has recently shown that an interchange chromosome with a B-centromere undergoes non-disjunction at the second pollen mitosis, while the interchange chromosome with an A-centromere behaves normally. The anomalous male transmission of intact B-chromosomes in maize (cf. p. 36) may also be accounted for on the basis of non-disjunction in the second mitosis (ROMAN, l. c.).

## SUMMARY.

Certain accessory chromosomes in rye show non-disjunction at the first division in the embryo-sac. The daughter chromatids are, as a rule, included in the micropylar nucleus. At the second and third divisions the accessories behave normally.

The presence of three accessories in a pollen cell or an embryo-sac causes a breakdown of the first mitosis. Deficient nuclei, supernumerary nuclei or less frequently a restitution nucleus are the result. When a spore has one accessory, mitosis is normal, when it has three accessories, the normal centromere-spindle mechanism is destroyed, while the presence of two accessories has an intermediate effect.

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# THE OAT CHLOROPHYLL MUTATIONS ALBOVIRESCENS, LUTEOMACULATA AND TIGRINA-1

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THE chlorophyll mutations mentioned were briefly reported before (FRÖIER, 1946). Owing to lack of space a more detailed account had to be postponed to the present volume of this journal.

## THE F. ALBOVIRESCENS OF HEXAPLOID OATS.

At the beginning of the growing season repeated, careful observations are annually made in the wheat and oat breeding fields at Svalöf. All larger field trial plots are then traversed, and notes made on sprouting in general, any possibly missing rows, damages by hares, and so forth. In addition, hand-hacking between the plot rows is almost always undertaken once or twice, perhaps three times, in the spring over the whole field. The skilled labourers have for the last 25 years had special orders immediately to report any observed chlorophyll-deficient oat or wheat plant. For these reasons it can be said that during the last 25 years there have been very small chances for chlorophyll-deficient forms segregating in the breeding field to have escaped observation. The measures of careful seedling control thus taken year by year have acted as an effective »sieve», capable of catching even slight chlorophyll deficiencies. A result of this incessant observation work during the last few years is the discovery of the f. *albovirescens* in the oat trials at Svalöf in 1939.

An $F_5$ progeny from a cross 01422 $b_2 \times$ 01431 b (for origin, vide p. 64) was sown in 1939 in two replications, No. 152—I and 152—II, each plot comprising an area of 15 square metres. In both plots plants were observed on the 3-leaf seedling stage which were entirely white except for the leaf-tip. The latter from the outset was very slightly yellowish-green (CDC = 296; »CDC» used as an abbreviation for »Code des Couleurs» by KLINCKSIECK et VALETTE, 1908); in later stages the tip greened more and more, and finally the whole leaf was green, never,

however, attaining the full-green of the normal neighbour plants (CDC = 311—312 for final *albovirescens* leaf stages; 326—327 for the normal neighbouring plant leaves of same order). Each new-developing leaf was at first white at the base and in the middle, the tip greening first. The plants were greatly retarded in growth. Only half of the number studied reached maturity as small, dwarf-like plants,

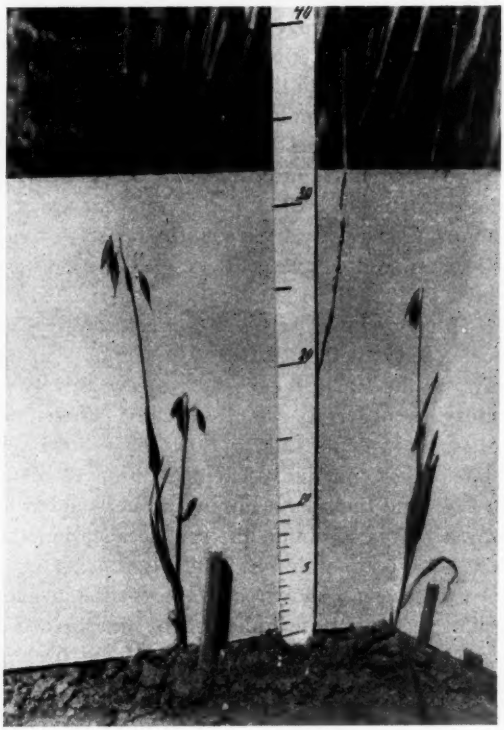


Fig. 1. *Albovirescens* plants from plot 1939—152—I. Scale numbers in centimetres.

setting 2 up to 5 (maximum recorded) seeds. The pollen was good. The seeds, though poorly developed, were capable of germinating and in the next season gave rise to constant *albovirescens* plants. Fig. 1 shows two such plants at harvest time in the plot 1939—152—I where they were first observed.

Each of the two plots mentioned above was estimated to contain about 3750 plants. In 152—I 15 *albovirescens* plants were counted; in

152—II nearly the same number were found, 14, just as in the former plot, uniformly distributed over the 15 square metres. 1 *albovirescens* plant was thus found per sq. m. The problem arose of how to isolate segregating progenies from the two plots, as it could be predicted that crosses with the supposed *albovirescens* recessives must be extremely difficult. If any higher segregation ratio than 3 : 1 caused the appearance of the dwarf plants, e. g., 15 : 1 or 63 : 1, care had to be taken to secure a great many plant progenies so as to get a sufficient number of segregating families represented. On the other hand, precautions had to be taken not to frustrate the purpose of the field trial, as a quite special interest from practical points of view was attached to the harvest results from the No. 152 in question. The writer took a middle course between these two viewpoints, and thus had 2 sq. m. harvested as single-plant progenies of each plot. 1029 plants were harvested, tied up carefully after being pulled up by the roots, in order to avoid losses and mixing-up of broken seed-carrying tillers.

In the winter of 1939 the 1029 plants were thrashed and each plant progeny divided in two equal parts. As a field cultivation of all 1029 plant progenies would have been difficult to carry out in practice, it was decided to grow out the 1029 progeny-halves successively in sand dishes in the laboratory in order to establish which progenies were really segregating ones. 13 plants out of 1029 proved to segregate *albovirescens* : green. The other halves kept of these 13 plants were sown in the field in 1940 and their segregation numbers added to those of the laboratory-grown halves to obtain the real segregation ratios of the 13 progenies. They are enumerated in Table 1.

The total segregations shown in Table 1 are in accordance with monomeric ratios, except those indicated by queries. The latter four progenies gave rise to the supposition that a di- or perhaps a tri-meric segregation lay behind the ratios found. By setting out the seed from all green plants next season in the field from the progenies 1940—957—1 to —13 it was, however, definitely established that this could not be the case, progenies 957—6, —7, —9 and —11 giving no segregation at all, which must necessarily have occurred if these progenies had been really di- or tri-merically segregating. All 3 : 1-segregating progenies showed a very regular repeated monomeric segregation : progenies 957—2, —3 and —5, for example, gave a total sum of 271 *albovirescens* : 832 green. The expected ratio would be $275{,}_9 : 827{,}_1$, i. e. a very close correspondence. No indications whatever of any embryonic (zygotic) lethality were found. Now, as regards the four progenies at first sup-

posed to segregate di- or even tri-merically, no other conclusion can be drawn than that some serious blunder must have been committed in the thrashing and dividing procedures; the four progenies in question must thus be eliminated from the discussion.

The recessive, however, was isolated from the 3 : 1-segregating progenies, and the experiments with growing the *albovirescens* plants in the greenhouse in order to get pollen in optimal time for field crosses during four years have wholly failed. Simultaneously, random crosses between green plants from 3 : 1-segregating progenies (among which the

TABLE 1. *Segregation of albovirescens plants from progeny halves, one half grown in the laboratory, 1939, the other half in the field, 1940.*

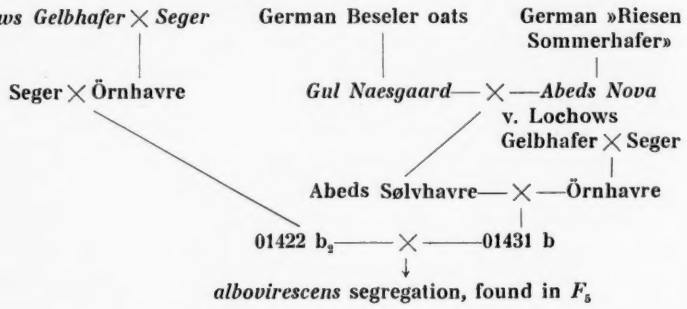
| Number of winter-grown divided plant progeny | Number of other half of the same plant progeny sown in the field | Segregation in winter-grown plants: | | Segregation in field-grown plants: | | Segregation in toto | | Probable segregation ratio |
|---|---|---|---|---|---|---|---|---|
| | | green | *albovirescens* | green | *albovirescens* | green | *albovirescens* | |
| 1939— 25 | 1940—957— 1 | 4 | 2 | 6 | 1 | 10 | 3 | 3:1 |
| — 49 | — 2 | 3 | 3 | 6 | 2 | 9 | 5 | » |
| —176 | — 3 | 5 | 4 | 7 | 3 | 12 | 7 | » |
| —192 | — 4 | 10 | 3 | 9 | 0 | 19 | 3 | » |
| —284 | — 5 | 8 | 1 | 8 | 5 | 16 | 6 | » |
| —537 | — 6 | 26 | 1 | 26 | 0 | 52 | 1 | ? |
| —554 | — 7 | 9 | 1 | 9 | 0 | 18 | 1 | ? |
| —558 | — 8 | 7 | 1 | 5 | 2 | 12 | 3 | 3:1 |
| —574 | — 9 | 27 | 1 | 29 | 0 | 56 | 1 | ? |
| —661 | —10 | 10 | 1 | 8 | 3 | 18 | 4 | 3:1 |
| —675 | —11 | 15 | 1 | 14 | 0 | 29 | 1 | ? |
| —771 | —12 | 10 | 7 | 14 | 5 | 24 | 12 | 3:1 |
| —900 | —13 | 10 | 7 | 12 | 5 | 22 | 12 | 3:1 |

heterozygotes will constitute the majority) have been tried on a large scale. Up to now, however, no success has been attained in these cross-experiments.

Is the new f. *albovirescens* thus isolated the result of an $F_2$ segregation following the bringing together of differently distributed polymeric *albovirescens* factors — cf. the discovery of the f. *lutescens* (ÅKERMAN, 1922) — or does it originate from spontaneous mutation?

To discuss these two possibilities, it seems appropriate to study the scheme of ancestral varieties of the two crossbred lines 01422 $b_2$ and 01431 b (Scheme 1).

SCHEME 1. *Ancestral varieties of the two crossbred lines 01422 $b_2$ and 01431 b.*


v. Lochows Gelbhafer × Seger
German Beseler oats
German »Riesen Sommerhafer«
Seger × Örnhavre
Gul Naesgaard — × — Abeds Nova
v. Lochows Gelbhafer × Seger
Abeds Sølvhavre — × — Örnhavre
01422 b2 — × — 01431 b
albovirescens segregation, found in F5

Some further particulars should be given as to the generations, preceding the $F_5$, in which the recessives were firstly observed. They are enumerated below:

Parents: 01422 $b_2$ × 01431 b. Cross made *1934*.

$F_1$: Two $F_1$ plants, the former yielding 278 kernels, the latter 106 kernels. *1935*.

$F_2$: Two $F_2$ progenies, both definitively not segregating any chlorophyll-aberrant plants. From the first one (*1936*—918 r 5) 57 plants were selected by ÅKERMAN, thrashed individually and thus yielding an

$F_3$: consisting of 57 progenies with 90 kernels sown per progeny (3 rows with 30 kernels each as usual in $F_3$) in *1937*. With the greatest probability no chlorophyll aberrants appeared in this $F_3$. A promising progeny, 1937—934 r 83, was selected, thrashed, and gave rise to an

$F_4$: comprising 3 replications, each 1 $^1/_2$ sq. m. Field No. *1938*—367. Just as $F_1$—$F_3$, $F_4$ was also sown with the aid of marking boards: in all, 1800 kernels were sown and gave rise to some 1200 seedlings. No chlorophyll-deficient plants were observed in this $F_4$; the writer himself made notes in the spring on these oat plots. After the harvest, about one-half of the seed was sown next year as an

$F_5$: in this $F_5$ the *albovirescens* plants suddenly appeared, *1939*.

In view of the particulars given above, the appearance in $F_5$ of the 15 + 14 *albovirescens* plants *must be ascribed to spontaneous mutation and not to segregation of polymeric factors.* In the latter case the totally green $F_2$ is large enough to rule out the possibility of a 15 : 1 segregation. A 63 : 1-segregation might by chance remain undiscovered;

such a trimeric segregation would, however, necessarily have caused a number of 15 : 1- and 3 : 1-segregating progenies to appear in $F_3$, which was not the case. Thus, spontaneous mutation either of a single gamete *Av* to *av* or — in case of vegetative mutation — of a primary meristematic cell *Av Av* to *Av av* must have taken place. A gametic mutation might at the first glance be supposed to have occurred in $F_3$; in such a case, however, the single resulting $F_4$ plant could not reasonably yield as many recessives (29) in $F_5$ as were found. If we assume that a gametic mutation occurred in $F_2$, the recessives may either have been overlooked in $F_4$, being only, say, 2—3 in number, or have by chance not segregated at all, the latter alternative being the more probable one. The number of recessives found in $F_5$ is then quite intelligible. As for the alternative assumption of vegetative mutation, such mutation must be supposed to have occurred in $F_3$, the recessives then not having been observed in $F_4$ or — more probably — not having segregated. An assumption of vegetative mutation in an $F_4$ plant cannot be maintained, since the number of recessives in $F_5$ is too large to permit tracing their origin from a single $F_4$ panicle or even a whole $F_4$ plant.

Reverting to the above scheme of ancestry (p. 64), it is of course of interest to continue the efforts of crossing the varieties printed in italics with *albovirescens* recessives (or the green monoheterozygote *Av av*) in order to determine whether polymeric inheritance exists even for this new *albovirescens* chlorophyll mutation. It may be added that chromosome counts on somatic metaphases from fixed root-tips of the recessives show a number of 42 chromosomes — just as in *chlorina* and *lutescens* recessives of hexaploid oats. Thus, no such abnormalities as those reported by PHILP (1935) in his *albina* type, with only 40 chromosomes, were found in these three forms.

Some anatomical and physiological observations have been made on the *albovirescens* recessives. At a rather early stage it was ascertained that *albovirescens* plants uniformly distributed in segregating field progenies and, being thus protected from more intense sunlight, did not show any distinctly accelerated degree of greening as compared with free-standing recessives. This independence of light distribution was also found in experiments with exposure of recessives to diminished light in the greenhouse. It is thus evident that the greening process of the chlorophyll apparatus in this recessive form is not perceptibly counteracted by light; on the contrary, it seems to be slightly favoured. This conclusion does not imply that chlorophyll-destroying reactions are not concerned with the mechanism of chlorophyll formation in

these *albovirescens* plants. This view is in full accordance with the plastid development studied during the ontogenesis.

Fig. 2 shows seedlings of the progeny 1939—49 (cf. Table 1) photographed 8 days after being put to germinate in wet sand; temperature 18° C. The three seedlings to the left are recessives; at the outset they were purely white, except for a faint yellow-green tinge

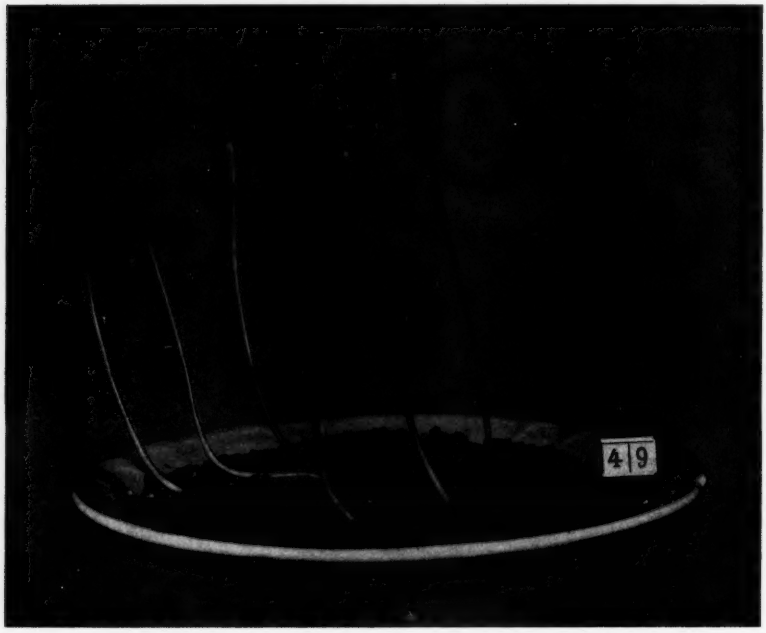



Fig. 2. Three *albovirescens* recessives (to the left) and three normal seedlings from the segregating winter-grown progeny 1939—49.

(CDC = 291) at the tip of the first leaf. The growing proceeded in full daylight in the laboratory. In Fig. 2 the slightly darker tinge of the tips of the *albovirescens* first seedling-leaves represents a light green colour, which rapidly deepens. At a length of 8 cm the tip of the first leaf (two days later than in Fig. 2) has the colour CDC = 302—303. The normal leaf has the colour CDC = 304. The base of the *albovirescens* leaf shows CDC = 261 (yellow-green), the base of the normal leaf CDC = 302—303 (pure green). Figs. 3 and 4 show sections of

normal-green and *albovirescens* leaves through tip and base of 10 days old seedlings.

Razor-cuttings in elder-pith, about 50—60 $\mu$ in thickness, have been made of the living material, the sections immediately being plunged into a 1 $^1/_2$—2 mol cane-sugar solution and then rapidly placed under the microscope. The drawings of the chloroplasts were made with a magnification of about 1050 $\times$. The same technique

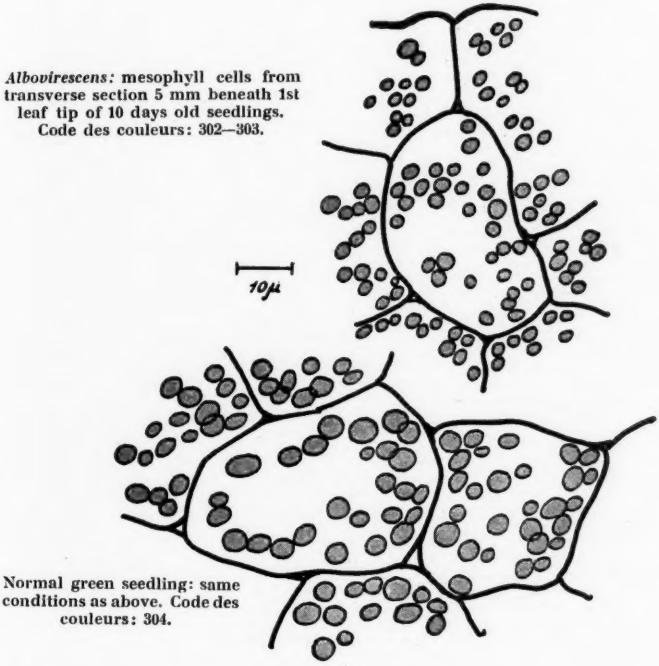

*Albovirescens:* mesophyll cells from transverse section 5 mm beneath 1st leaf tip of 10 days old seedlings. Code des couleurs: 302—303.
10μ
Normal green seedling: same conditions as above. Code des couleurs: 304.

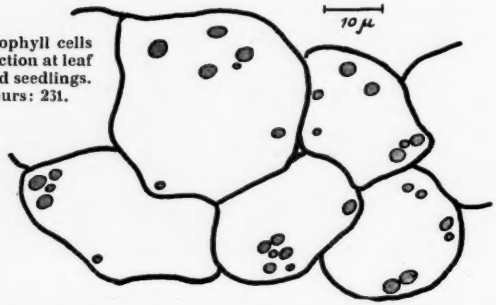
Fig. 3. Transverse sections of normal-green and *albovirescens* plant leaves. Mesophyll cells with plastids from leaf-tip of 10 days old seedlings.

was adopted by GUSTAFSSON (1942) in studying plastid structure in barley chlorophyll mutations (cf. his figures; l. c., p. 487). That paper contains a critical review of earlier, rather scarce, chloroplast studies on chlorophyll-mutant forms with exhaustive literature references, which will therefore be omitted here. The experiences of the present writer entirely coincide with those of GUSTAFSSON in regard to the use of strong fixatives. These fluids almost always alter the natural shape and size of the plastids considerably, and they cause these granules to clump together, so that no reliable comparisons, e. g., between aberrant and normal-green form can be undertaken.

Fig. 5 shows sections of 5th leaf of normal and *albovirescens* plants at the stage when the first panicle has just developed. All figures show medium-sized mesophyll cells.

The plastid ontogeny of the *albovirescens* recessives may be summed up as follows. The first leaf of the young seedlings shows only faint signs of chlorophyll. At a stage when the first leaf has just

Normal green plants: same conditions as below. Code des couleurs: 302—303.

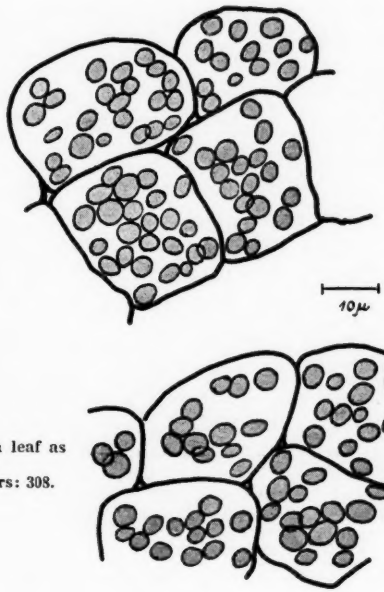



*Albovirescens:* mesophyll cells from transverse section at leaf base of 10 days old seedlings. Code des couleurs: 231.

Fig. 4. Transverse sections of normal-green and *albovirescens* plant leaves. Mesophyll cells with plastids from leaf-bases of 10 days old seedlings.

ruptured the coleoptile sheath, sections of mesophyll cells show small but undoubtedly green to yellow-green plastids and partly as yet non-coloured proplastids; among the green plastids some have already attained a considerable size. At a later stage (Fig. 4) the still whitish-green leaf base shows cells where some of these large plastids have about the same size as those of fully normal-green plants from the same basal section. The difference in plastid number is, however, very

marked, as there is only about $^1/_3$—$^1/_4$ of the normal plastid number present in the cells of the aberrant form. At the tip of the first leaf, on the other hand (Fig. 3), the *albovirescens* plastids at this time are numerous, so that it is not possible to establish any significant difference in number from the normal plants. Even 10 days after germination, however, the average size of the plastids does not attain that of the normal ones: equally large plastids are developed only in later stages




Normal green plant: 5th leaf mesophyll cells in transverse section.
Code des couleurs: 327.

*Albovirescens:* 5th leaf as above.
Code des couleurs: 308.

Fig. 5. Transverse sections of normal-green and *albovirescens* plant leaves. Mesophyll cells with plastids from the 5th leaf of plants with new-developed panicles.

than those illustrated in Fig. 3. The green colour of both leaf-tips at the 10-day stage is only slightly different (see figure-texts). Each subsequent leaf in the plant ontogenesis goes through these plastid development stages, although relatively faster for each new leaf. Thus, at the 5-leaf stage, when panicles begin to emerge, sections show that it is no longer possible to find any difference either in size or number of plastids between *albovirescens* leaves and normal ones. The real difference from now on consists merely in the deepness of the green colour, the normal plants rapidly advancing towards dark, vivid green. A small

tabulation may serve to illustrate the general colour appearance of both kind of plants at the 5-leaf stage (colour according to CDC):

| Leaf order | *Albovirescens* recessive | Normal-green plant |
|---|---|---|
| 5th leaf | 308 (medium green) | 327 (dark green) |
| 4th » | 288 (dark yellow-green) | » » » |
| 3rd » | 282 (medium yellow-green) | » » » |

The 1st and 2nd leaves at this stage were too withered for colour determination. From the above table it can be seen that the leaves of lower (earlier) order do not attain a full normal-green colour (CDC = = 301—350), but remain more or less yellowish-green on the major area of the leaf, the tips, however, regularly attaining a colour of 302 up to 308, i. e. normal-green.

The *albovirescens* aberration entirely coincides with GUSTAFSSON's subgroup *viridoalbina* and would have been designated according to GUSTAFSSON but for its discovery before his definite classification was published, the present writer then having already given a primary report on the new mutation (ÅKERMAN and FRÖIER, 1941, p. 372).

Finally, a few observations on temperature influence on chlorophyll development of the recessives should be reported. At temperatures of + 6° to + 8° C. the green colour of the successively developing leaves does not reach the same intensity and develops much more slowly than is the case at temperatures of about + 15° to + 18° C. Thus, in laboratory or greenhouse cultures the first leaf of *albovirescens* seedlings is capable of producing chloroplasts in the tips of nearly, if not exactly, the same size and number as in normal plants at the same stage. Summer temperatures of 25° C. to 30° C. in the greenhouses cause a strong acceleration of the greening of the *albovirescens* leaves, compared with the same developmental stages observed in the open field at 20°—22° C.

## THE F. LUTEOMACULATA OF HEXAPLOID OATS.

The f. *luteomaculata* was discovered in 1937 by Professor ÅKERMAN and Miss H. JAKOBSSON in a small plot of élite *b* of the oat strain Guldregn II (from Seger × Guldregn I; ÅKERMAN, 1928). Among 60 seedlings 2 were observed that displayed yellow or yellow-green spots irregularly distributed over the second and third seedling leaves (Field No. 1937—948$_1$). As was afterwards ascertained, the CDC for these spots averages 281—283, the normal leaf colour at this stage measuring 301—303. The new form was handed over to the present writer for

closer study. In the course of some six years' subsequent observations it has been ascertained that the first seedling leaf merely to a minor extent exhibits such spots or areas of yellowish colour, and that the fourth leaf and leaves of later order are quite normal-green during their development. In Fig. 6 normal and *luteomaculata* seedlings in the 3-leaf stage have been photographed with Ilford Panchromatic plates and a dense filter.

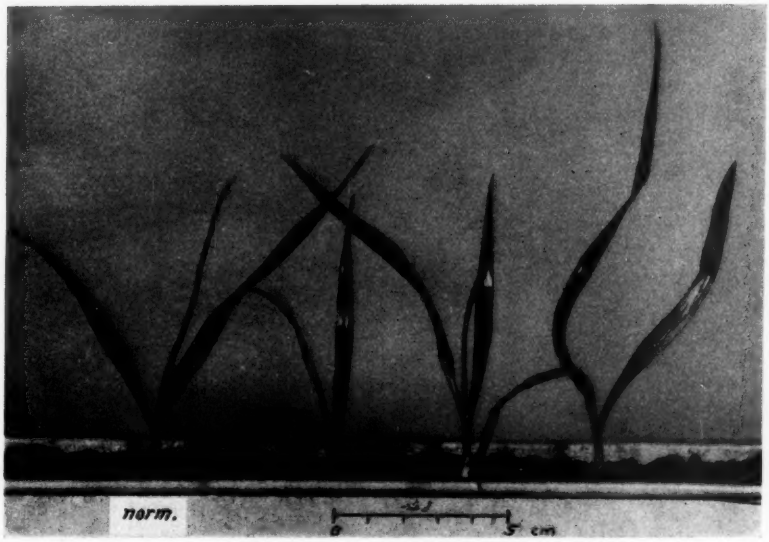



Fig. 6. Normal (to the left) and *luteomaculata* seedlings at 3-leaf stage.

Selection experiments 1938—1941 (Fig. 7) show that the progenies of typical *luteomaculata* plants are quite constant. In the first year of progeny observation (1938), however, a few mistakes were made in the classification of the aberrant plant type. Hence three plants in 1938 were — with some hesitation — classified as normal-green; the next generation showed them to be typical *luteomaculata* plants. Edaphic conditions seem to have a certain influence upon the development of the *luteomaculata* character, since plots situated on parts of the field with less soil humidity or on parts mixed up with portions of the subsoil have shown larger areas of spotting in the *luteomaculata* plants than have plots on richer and more humid parts of the field.

In 1940 and 1941 special care was taken in the classification and study of the *luteomaculata* plant progenies, this in order to determine the homogeneity of these progenies and their thorough identity with the mother strain Guldregn II. So far, no differences between the

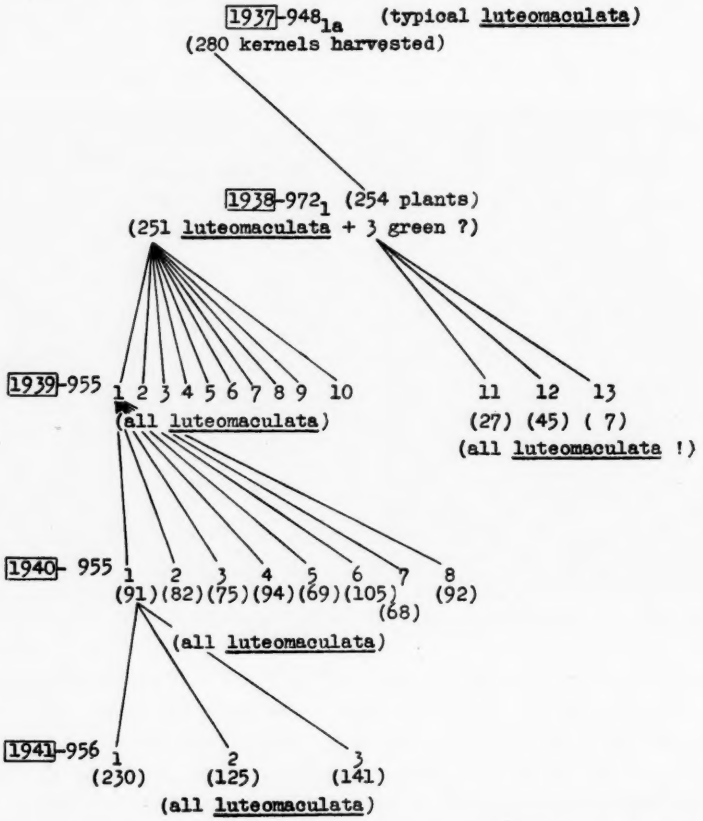



Fig. 7. Selection experiments with the f. *luteomaculata* 1937—1941. Figures in brackets = number of seedlings per progeny.

f. *luteomaculata* and Guldregn II except in chlorophyll development have been observed. No reversions from *luteomaculata* to normal-green were noted. In order to study the inheritance of the f. *luteomaculata*, reciprocal crosses were made with Guldregn II (*01221 b*) in 1938—1940. The results are given in Table 2.

From Table 2 it must be concluded that the inheritance of the *luteomaculata* character is non-mendelian and exclusively maternal. Quite special care was taken in these two cross-combinations: the emasculations were carried out at a somewhat earlier stage than usual — under the writer's own supervision — so that the possibility of selfing may be

TABLE 2. *$F_1$ and $F_2$ from reciprocal crosses between f. luteomaculata and Guldregn II (01221 b).*

| Number and year of cross | Combination | $F_1$ | | | $F_2$ | | |
|---|---|---|---|---|---|---|---|
| | | Field number | *Luteomaculata* | Green plants | Field number | *Luteomaculata* | Green plants |
| 335 in 1938 | Guldregn II × *luteomaculata* | 1939—958—54 | — | 3 | 1940—951—61—63 | — | 357 |
| 338 in 1938 | » | 1939—958—56 | — | 1 | — | — | — |
| 162 in 1940 | » | 1941—950—20 | — | 2 | 1942—941—1—2 | — | 589 |
| 163 in 1940 | » | 1941—950—22 | — | 2 | 1942—941—3—4 | — | 595 |
| 164 in 1940 | » | 1941—950—24 | — | 4 | 1942—941—5—8 | — | 732 |
| 165 in 1940 | » | 1941—950—26 | — | 4 | 1942—941—9—12 | — | 760 |
| 166 in 1940 | » | 1941—950—28 | — | 7 | 1942—941—13—19 | — | 1379 |
| 167 in 1940 | » | 1941—950—30 | — | 3 | 1942—941—20—22 | — | 674 |
| 101 in 1939 | *Luteomaculata* × Guldregn II | 1940—950—1 | 1 | — | 1941—952—1 | 130 | — |
| 103 in 1939 | » | 1940—950—2 | 5 | — | 1941—952—2—6 | 1070 | — |
| 104 in 1939 | » | 1940—950—4 | 4 | — | 1941—952—7—10 | 898 | — |

regarded as ruled out. It should be stressed that in this special cross (and its reciprocal) it is utterly impossible to make any morphological $F_1$ control. The conclusiveness of the experiments recorded above thus implies the thorough success of the crosses as a *sine qua non*.

In the writer's opinion, the non-mendelian inheritance of the f. *luteomaculata* can be interpreted according to the classical scheme of CORRENS (1908) for his *albomaculata* type, afterwards discussed by

GREGORY (1915), WINGE (1919) and CORRENS (1931). This hypothesis requires that the »sick» plastids shall be transmitted solely by the egg-cell. In the *luteomaculata* case, however, no assortment of »sick» and »normal» plastids has as yet been observed in any progeny studied. The works of RENNER (1934, 1936) on the genetic autonomy of the plastids (see these papers for comprehensive surveys of the literature on inheritance of variegation) give good *reasons to assume a previous plastid mutation* (»Plastom»-Mutation in RENNER's terminology) as causing the appearance of the f. *luteomaculata*. There is, unfortunately, no possibility of tracing this assumed plastid mutation to a certain generation, as the necessary observations on the generations next to the 1937-generation are missing. The 1937-generation originates from seeds three years old (harvest 1933); the next preceding generation was grown in 1929. The seed-ageing may perhaps have afforded greater possibilities for plastid mutation; cf. the results reported by SCHKWARNIKOW (1937) in wheat and by PETO (1933) in maize.

It is interesting to find that this exclusively maternal inheritance of the *luteomaculata* character manifests itself regularly also in reciprocal crosses with the hexaploid oat chlorophyll mutation f. *chlorina* (cf. ÅKERMAN and FRÖIER, 1941) from Guldregn I. Such crosses were made in large number in 1940. From the cross *chlorina* × *luteomaculata* 11 $F_1$ plants were procured, all typical *chlorina* without the slightest signs of *luteomaculata* spots. The $F_2$ in 1942 from these 11 $F_1$ plants consisted of 11 quite regularly 3 : 1-segregating progenies (in total: 1676 distinctly normal-green and 531 distinctly typical *chlorina* recessives; the theoretical numbers would be 1655,2 to 551,8 with $\chi^2 = 1{,}046$ and $0{,}50 > P > 0{,}30$). From the reciprocal cross *luteomaculata* × *chlorina* 2 $F_1$ plants were procured, both typically *luteomaculata* spotted on 2nd and 3rd leaf. The two $F_2$ progenies grown in 1942 segregated regularly 3 : 1 (in total 171 *luteomaculata* spotted to 52 *chlorina with the luteomaculata spotting superimposed*; the expected numbers would be 167,2 to 55,8 with $\chi^2 = 0{,}345$ and $0{,}70 > P > 0{,}50$). The first of these $F_2$ progenies segregated in 105 *luteomaculata* : 35 *chlorina* with superimposed *luteomaculata* spotting; an $F_3$ was grown from 10 of the *luteomaculata* plants and from 10 of the »*chlorino-luteomaculata*» plants. This $F_3$ was grown in the field in 1943 under favourable conditions for the *luteomaculata* dots to develop. The former 10 $F_3$ progenies consisted of 5 regularly 3 : 1-segregating progenies (in total: 240 *luteomaculata* to 91 »*chlorino-luteomaculata*»; expected for 3 : 1 would be 248,2 : 82,8) and of 5 constant *luteomaculata* progenies. The

latter 10 $F_3$ progenies consisted only of »*chlorino-luteomaculata*» plants. Thus, the strict persistence of the maternally inherited *luteomaculata* character was obvious, even introduced in another genetic environment causing a rather marked chlorophyll deficiency.

The anatomical study of the pregnant yellow spots in *luteomaculata* plants reveals an interesting feature. The spots are as a rule best developed on the second leaf and especially manifest when the seedlings (granted normal field conditions) are about 15 cm high, and the third leaf is just emerging. Fig. 8 shows a transverse section of mesophyll

Fig. 8. F. *luteomaculata*: transverse section of living mesophyll cells from yellow spots of the second leaf from seedlings, 15 cm high.

cells from such a distinct yellow spot. Among fully green cells with normally developed chloroplasts, cells occur where the chloroplasts seem to be in a state of dissolution (Fig. 8). All transitional stages from normal-green distinct plastids through more or less »dissolved» ones down to completely destroyed plastids can be observed in the same cell. In roughly 30—40 % of the cells with degenerated plastids a few (1—5) chloroplasts still seem to persist. Cells even with totally degenerated plastids do not perceptibly lose their turgor. Therefore, in the yellow spots we never find any »shrunk» features of the leaf — this in contrast, e. g., to the behaviour of the rare members of the *tigrina* group in barley, oats (cf. p. 80), and the »zebra-striped» forms in maize. The *tigrina* types show a more advanced necrosis, the degenerating cells

losing their turgor and being squeezed together with transverse leaf area deformations as the result.

Leaves of higher order than the third do not seem to display any distinct signs of this plastid degeneration. The second leaf exhibits them most clearly: even up to the withering stage the second leaf is as a rule recognizable as yellow-spotted, although the spots are less conspicuous and the green colour much deepened. In the writer's opinion this colour deepening of the yellow spots must be explained as follows. Colourless proplastids, present in the necrotic cells but practically impossible to recognize in sections of living tissue partly give rise to new, chiefly normal, chloroplasts. This assumption is supported by the observation that later stages of second leaf yellow spot development towards deeper green show up to 80—90 % mesophyll cells with some few distinctly green plastids together with the »dissolved» ones, while 20—10 % of the mesophyll cells show only »dissolved» plastids. Hence the deeper colour of the later stages of the yellow spots on the second leaf.

Previously reported studies on similar chloroplast degeneration in grasses seem to be scarce. The »blotch leaf» of FERDINANDSEN and WINGE in oats (1930) was never studied in regard to plastid structure. RANDOLPH (1922), however, among chlorophyll-aberrant maize forms also examined a maternal inheritance strain and, summing up, found that the aberrations were due to the failure of the proplastids to develop into plastids of normal size, or colour, or both. ZIRKLE (1929), from similar studies on maize, concluded that the plastid primordia were normal, but that the phenotypes of different aberrations were the result of a delayed plastid development, an inhibition of development, or an inhibition followed by degeneration.

## THE F. TIGRINA-1 OF DIPLOID OATS.

In 1935 ÅKERMAN found a »spotted» plant type in a control plot of untreated *Avena strigosa* SCHREB. ($2n = 14$) belonging to an experiment series with X-raying of diploid oats. The *strigosa* line originated from a pedigree selection of a land oat from Hinneryd, Småland, Sweden. It is well-known that *A. strigosa* often occurs as a weed component in many land oats of this province (cf. GRANHALL, 1938). This Hinneryd *strigosa* line was observed in Svalöf for several years after the primary pedigree selection in 1930.

The »spotted» plants, which have undoubtedly arisen by spontaneous

mutation, were studied by the present writer from 1938 to 1945. They are characterized by very obvious transverse strips or dots, especially

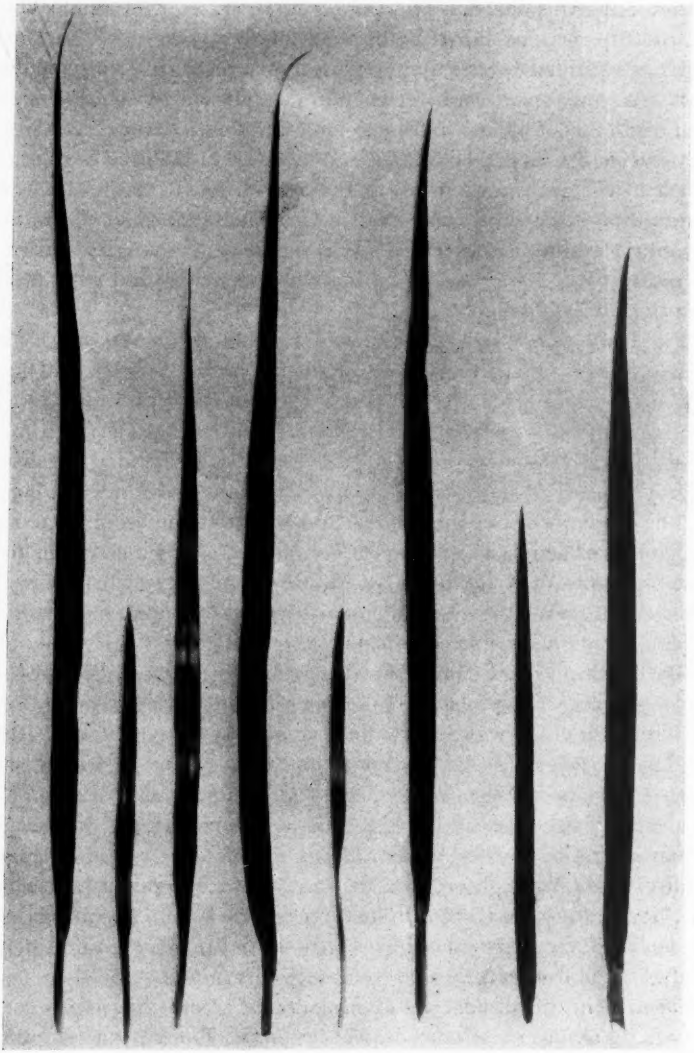


Fig. 9. *Tigrina-1* leaves (the left five) and normal (the right three) leaves. Seedlings 25 days old. See text.

on the first seedling leaf but also quite manifest on the second leaf. Leaves of higher order do not as a rule show these bands. The further development of the plants is retarded, the panicles are small, and few seeds produced. The pollen is good. Chromosome counts from root-tip sections show the regular number, 14.

The striped leaves give an impression of several differently coloured zones (hence the aberrant form was at first called »variozonata»), which could be defined according to Code des Couleurs: green 303—304, yellow-green 286—287, brownish, red-violet 557—558. The mutation belongs to the rare mutant group *tigrina* (cf. GUSTAFSSON, 1940) and should properly be called *tigrina-1*. (As to the index -1 of *tigrina*: The chlorophyll mutations described are designated in numerical order for each oat species. X-ray induced mutations are symbolized with an »X»; hence *tigrina-X-1* and so forth).

Fig. 9 shows to the left five leaves from the *tigrina-1* recessive (the two short ones first leaves, the three others second leaves), and to the right three leaves from normal-green seedlings of the same age. On the 4th leaf from the left transverse leaf area contractions of the discoloured zones are perceptible. This feature is accounted for by the anatomical study of sections through the aberrant leaf zones (cf. below).

The development of the 1st and 2nd leaf zonation seems practically independent of edaphic and climatic conditions. Growings in the greenhouse in diminished light (one-third of normal daylight) show less anthocyanine development, and brownish zones are not met with; for the rest, zonation develops are usual.

By causing green plants from the harvest of 1937, taken from the *tigrina-1*-segregating plots 1937—951 r 58 and 68 to repeat the segregation, the latter was shown to be regularly monofactorial. Thus, in 1938 the total segregation numbers from 14 progenies were 219 *tigrina-1* : 671 normal-green (the expected values would be 222,5 : 667,5, i. e. a very close correspondence). Also in following years quite regular monomeric ratios were obtained. No signs of any zygotic or gametic lethality have been observed. In years with severe spring drought (e. g., 1941) the deficit of *tigrina-1* recessives is but slightly marked. The *tigrina-1* recessives have been crossed with other *Avena strigosa* varieties as an attempt to ascertain whether perhaps other *tigrina* factors exist in this diploid oat species. Such crosses have been carried out with *A. strigosa* v. *glabrescens* MARQUAND (THELL.) and *A. strigosa* ssp. *pilosa* v. *alba* MARQUAND (cf. MARQUAND, 1922, and JONES, 1931, as to taxonomy; both varieties from Wales). These varieties, procured

from Aberystwyth by the courtesy of the Deputy Director, Dr. R. O. WHYTE, proved, however, to give a fully regular monomeric segregation when crossed with the *tigrina-1* recessive.

The anatomical study of the *tigrina-1* recessives reveals an advanced necrosis of chloroplasts not hitherto described in any studied oat or wheat chlorophyll aberration. In Fig. 10 a transverse leaf section is shown (the original drawing was made in 340 × magnification); it will be seen that certain mesophyll cell groups or isolated single cells have totally degenerated chloroplasts. Only diffuse masses, strongly anthocyanine coloured, are observed in most of these cells. The cell-complexes thus characterized have partially or wholly lost their turgor.

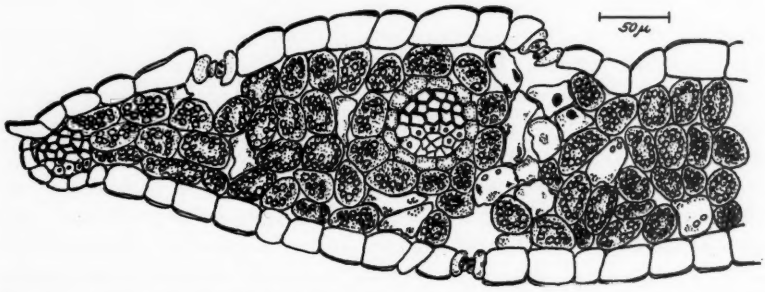



Fig. 10. Transverse section of a coloured zone of a leaf from *tigrina-1* recessive homozygote of *A. strigosa* (first seedling leaf; length 7 cm; seedlings 25 days old).

Hence the surrounding normal cells press these low-turgor cells together, resulting in sunken longitudinal strips of the leaf and a transverse contraction of the leaf area over the whole zones. The epidermis cells partake passively in this shrinking of the leaf; in Fig. 10 the upper part to the extreme right clearly illustrates such a »sunken strip» in transverse section. Transitional stages of plastid decomposition are found; a few obviously normal chloroplasts of pure green colour may still be found in otherwise fully degenerated cells. There is no definite location in the mesophyll of the decomposed cells. They occur scattered over the whole leaf area, but as a rule are concentrated in groups. Stomatal cells have not been found to degenerate in this manner.

The *tigrina-X* mutations (induced by X-raying) in barley (cf. GUSTAFSSON, 1940) — no spontaneous mutations of this type have as yet been found in barley — are related to the zebra-striped types in *Zea mays* and to the *tigrina-1* spontaneous oat mutation described. The

barley *tigrina-X* types are not viable in contradistinction from the zebra-striped types in *Zea*, which are only slightly subvital. DEMEREC (1921) discovered the first zebra type: $zb_1\ zb_1$. This type showed a pronounced »zebra» character only in mature plants and quite faintly, or not at all, in seedling stages. DEMEREC (l. c., p. 407) states that »zebra striped leaves are wavy probably because of unequal growth of the green and chlorotic tissues». No anatomical study of the cells was reported, but the »wavy» appearance could most probably be ascribed to partial necrosis connected with loss of turgor in essentially the same manner as shown above for *tigrina-1*. The next zebra type in maize was reported by STROMAN (1924): $zb_2\ zb_2$. Here the character appeared only in the seedling stage and then totally disappeared. In a later paper DEMEREC (1924) briefly states that intercrosses have suggested the existence of 4 different genes for zebra seedlings in maize. He designates them $ze_1$—$ze_4$. Finally, HAYES (1932) reported another zebra type, displaying its characters only in the 2- and 3-leaf stage. Before maturity the transverse striping entirely disappears. Certain temperature conditions are necessary for the development of the striping.

The *tigrina-1* mutation in diploid oats thus holds an intermediate position between the lethal barley *tigrina-X* types, on the one hand, and the only slightly subvital zebra types in *Zea mays*, on the other. It is also of interest to note that solitary *tigrina-X* mutations have been procured from both X-rayed diploid wheats (L. SMITH, 1939, p. 18: *yz yz*, i. e. »yellow-zoned» — as the present writer wishes to interpret this mutation), and diploid oats (cf. FRÖIER, 1946, Ch. V). The wheat mutation, procured by SMITH, is quite vital; the induced oat mutant of *A. strigosa* (*tigrina-X-1*) is distinctly more viable than the spontaneous *tigrina-1* mutation. It may be added that a *tigrina-X-1* mutation from the diploid *A. brevis* (FRÖIER, 1946) and two *tigrina-X* mutations found in hexaploid oats (l. c.) are lethal-sublethal.

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# THE EFFECT OF COLCHICINE AND ETHYLENE GLYCOL ON STICKY CHROMOSOMES IN ALLIUM CEPA

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IN another paper, actually in preparation, I will report some observations made during the period October 1945—April 1947 when using the Feulgen nucleal stain (FEULGEN and ROSSENBECK, 1924) with different plant materials and techniques. A study of mitotic chromosome structure, stickiness and related phenomena in *Allium Cepa* as well as a discussion of the problem will appear on that occasion.

The present paper deals with the effect of colchicine and ethylene glycol on sticky chromosomes in *Allium Cepa*, representing the last part of my investigation upon stickiness in the root-tips of this plant. The work was carried out with the purpose of reproducing, in another way, some of the cytological phenomena that have been recently reported by many authors when studying the action of colchicine and other c-mitotic compounds both in plants and animals (BERGER and WITKUS, 1943; BARBER and CALLAN, 1943; BERGER, WITKUS and SULLIVAN, 1944; WITKUS and BERGER, 1944; LEVAN 1945; VAARAMA, 1947; NYBOM and KNUTSSON. 1947).

## I. METHODS.

The possibility of experimentally producing sticky chromosomes is known since GALEOTTI's work (1893). Since then different physical and chemical agents have proved to be effective for this purpose, namely (a) chemicals (YAMAHA, 1927; WITKUS and BERGER, 1944; ÖSTERGREN, 1944 b; LEVAN, 1945; VAARAMA, 1947; NYBOM and KNUTSSON, 1947); (b) radiations (see literature in POLITZER, 1934 and LEA, 1946); (c) high (e. g., MILOVIDOV, 1932 and SAX, 1937) and low temperatures (DARLINGTON and LA COUR, 1940; GEITLER, 1940).

In this last case interesting features are to be detected in the chromosomes of some plants as a consequence of a »nucleic acid starvation».

During my experiments on *Allium Cepa* an artificial stickiness was attained with different and sometimes simple methods. But the bulbs

used for this investigation belong to the same lot of seemingly untreated material from which a full study of chromosome structure and stickiness was derived. These bulbs happened to have been stored in a storehouse where minimum temperatures ranging from 0° to —4° C. were present.

Probably low temperatures, acting for a long period of time, have been able here to produce a physiological effect, the result of which was to induce sticky chromosomes. On immersion of their basal portions in water at room temperature the root-tips obtained from these bulbs show only a moderate degree of stickiness, i. e. stickiness not hindering metaphase congression and anaphase separation of the chromosomes. Moreover, as shown by observations actually in progress, stickiness of chromosomes in this material seems to be — at least to some extent — a vital condition, many plants obtained from these bulbs in pots continuing life and growth under the greenhouse conditions.

For the colchicine experiments a 0,01 % solution was employed and treatments for 4, 8 and 12 hours were made. For the ethylene glycol treatment two different samples were used, namely ethylene glycol S 1767 KEBO, Stockholm, and ethylene glycol 1316 Riedel and de Haën, Berlin; 4 mol, 2 mol, 1 mol and $^1/_2$ mol solutions being employed and the exposures lasting for 4 hours, 8 hours, 12 hours, 1 day and 3 days. For each series of experiments untreated root-tips of the same bulb served as controls. Fixing in La Cour's 2 BD took place soon after each treatment. Most material was prepared by the smear technique after the Feulgen reaction (HEITZ, 1936): fixation lasted 30′—180′; and hydrolysis — in N/HCl at 60° C. — from 6′ to 10′. Bleaching in a 3 % solution of $H_2O_2$ was applied after staining in leuco basic fuchsin. The slides were all made permanent in Canada balsam according to HILLARY (1940). For comparison and especially critical purposes, root-tips subjected to the various treatments, fixed in 2 BD and prepared by the ordinary paraffin technique, were cut into longitudinal sections and stained in crystal violet. From a comparative analysis of the cytological phenomena both in smears and sections, I am able to infer that the smear method is very effective in studying such problems as those dealt with in this paper and in some respects is to be preferred to the ordinary section technique.

The microphotos were taken with a Zeiss »Vertikalkamera Standard» on Agfa Isopan plates. A combination of a violet and a yellow filter was exclusively employed.

## II. THE EFFECT OF COLCHICINE.

### 1. COURSE OF THE C-MITOSIS.

A full c-mitosis with complete spindle inactivation and c-pairs in all the cells was observed only after the 8 and 12 hours' treatments. After the 4 hours' treatment full c-mitosis, partial c-mitosis (ÖSTERGREN, 1944 a) with partially functional and tripolar spindles and completely normal anaphases were to be seen in the various cells of the same root-tip.

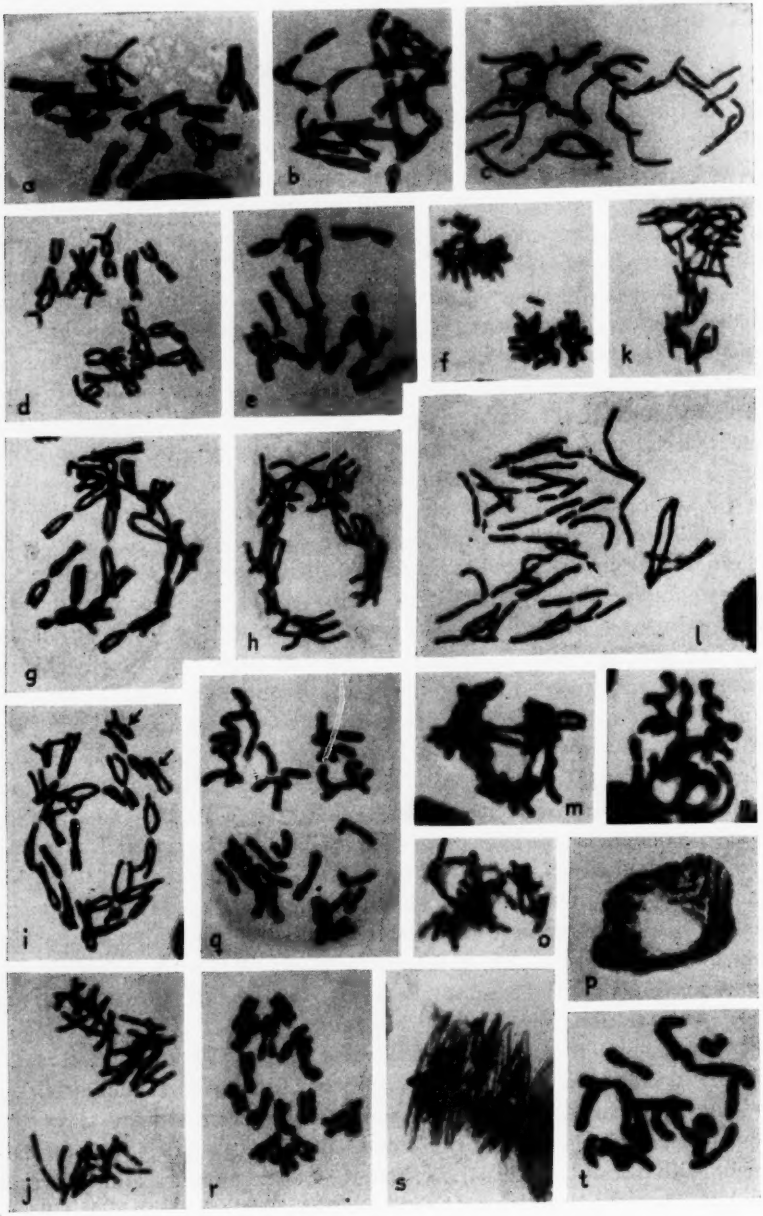
In the case of a partial c-mitosis sticking of the chromosomes, impeded anaphase separation and lack of cell-wall formation are responsible for the frequent appearance of bi-nucleate cells. Sometimes a fusion of the two anaphasic chromosome groups occurred before and after reversion to the resting stage. In other cases large bridges joining the two telophase nuclei or two telophase nuclei with and without micronuclei in the same cell appeared.

Exceptionally the normal anaphasic separation failed both as a consequence of stickiness and of spindle disturbances. A nearly constant feature in the telophase nuclei is their very irregular and sometimes thorny shape. Nuclei of a similar appearance occur, although rarely, in the controls. This suggests an influence by the drug on the nuclear membrane, as first pointed out by BHADURI (1939). Colchicine seems also responsible for the much more frequent occurrence of micronuclei in the cells exposed to its action as compared with the controls.

The above-mentioned phenomena were first detected in *Allium Cepa* by WITKUS and BERGER (1944) after treatment with veratrine, a new polyploidy inducing agent, also effective in the induction of sticky chromosomes. It seems probable that other compounds showing a similar double action, such as inorganic salt solutions (LEVAN, 1945) and methyl naphthoquinone (NYBOM and KNUTSSON, 1947), in some definite ranges of concentration and exposure, induce similar cytological phenomena. Benzene (BERGER, WITKUS and SULLIVAN, 1944), in my opinion, could also be included in this list, although stickiness does not seem to have been observed by these authors.

The complete series of stages reported by WITKUS and BERGER (1944) in their photomicrographs G-N was observed in my material.

When a full c-mitosis is present, the c-pairs generally show the appearance first described by WITKUS and BERGER (1944) after treatment with veratrine, i. e. »the chromatids do not separate and diverge widely, forming the x-shaped diplos characteristic of colchicine treat-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ment». This must be attributed, as WITKUS and BERGER do, to the »sticky» condition of the chromosomes. But, especially after the 4 and 12 hours' treatments, typical X-shaped c-pairs (LEVAN, 1938) can also be observed, as well as all the intermediate shapes. According to ÖSTERGREN (1943), the »elastic repulsion» between the two chromatids is responsible for the peculiar appearance of the c-pairs. When stickiness is acting in different regions of the chromosome body, the shape assumed by the c-pairs must evidently be the result of two forces, viz. elastic repulsion localized at the centromere and traction between the chromatids at the sticky regions. Fig. 1 *a* gives a clear picture of this phenomenon. In Fig. 2 the 16 c-pairs have been separately drawn in order to make evident the various sticky regions (dotting); constrictions and centromeres are very clear, thus confirming the possibilities of studying chromosome morphology after treatment with colchicine, as first suggested by O'MARA (1939). In the SAT-pair of Fig. 2 *h* an asymmetrical distension of the SAT-thread is visible. This phenomenon is already known in the cytological literature and has been described at different times, e. g., by RESENDE (1937, 1941; 1946 in collaboration with DA FRANCA). In some cases asymmetrical distention is a consequence of stickiness between satellites; but it does not seem to be always so. Further details will be given in my later paper. Stickiness acts also between different c-pairs; especially their terminal portions and SAT-zones stick together, giving chains of 2, 3, 4, and sometimes 5 c-pairs. This is particularly evident after the 8 and 12 hours' treatments in relation to peculiar distributions in the cell cavity. Thus in Fig. 1 *b*, showing a c-metaphase after treatment for 12 hours, a terminal sticking of 3 chromosomes — one of which a SAT-chromosome — is

Fig. 1. *Allium Cepa.* Effect of colchicine and ethylene glycol on sticky chromosomes. Magnification of all microphotos × 1000. — *a—p*: 0,1 % solution of colchicine. — *a*: C-metaphase; 4 hours' treatment. — *b*: Terminal sticking of three c-pairs; 12 hours' treatment. — *c*: C-anaphase; 4 hours' treatment. — *d—e*: »Distributed» c-metaphases; 8 hours' treatment. — *f*: »Pseudo-anaphase» with a fragment; 8 hours' treatment. — *g—i*: Arrangement of the c-pairs around the achromatic sphere; in *i* misdivision of a c-pair is pictured; 12 hours' treatment. — *j—k*: »Distributed» and typical c-anaphase respectively; 8 hours' treatment. — *l*: C-anaphase with two chromosome arms or acentric fragments (arrows); 12 hours' treatment. — *m*: Precocious reversion in a tripolar anaphase; 4 hours' treatment. — *n*: Precocious reversion in a condition of »ball-metaphase»; 8 hours' treatment. — *o*: Precocious reversion before splitting of most c-pairs; 8 hours' treatment. — *p*: Restitution nucleus around the achromatic sphere. — *q—t*: Ethylene glycol in various concentrations. — *q—r*: Multipolar spindle and c-anaphase; 1 mol solution for 12 hours. — *s*: »Prophasic metaphase»; 1 mol solution for three days. — *t*: Arrested metaphase (probably ball-metaphase); 2 mol solution for 4 hours.

very evident, the sticking of this chromosome to the other two being insured by one of the two satellites.

C-pairs, scattered over the cell and presenting a normal development to the division of the centromere and the production of tetraploid cells, are a constant feature in the material treated for 4 hours. The traction exerted by stickiness during c-anaphase seems to be responsible for some peculiar configurations (Fig. 1 *c*). After the 8 and 12 hours' treatments this normal course of the c-mitosis can be observed only in a few cases. Generally new forces seem to enter into action during c-metaphase, causing »distributed c-mitosis» (NYBOM and KNUTSSON,

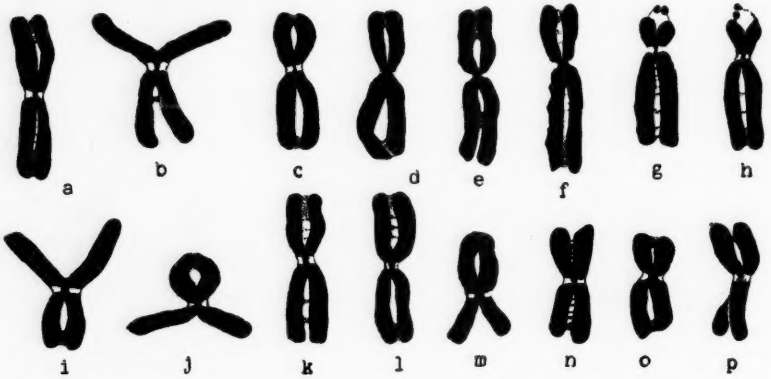



Fig. 2. The 16 c-pairs of Fig. 1 *a* separately drawn. Dotting shows sticky regions. — × 2200.

1947), and some pictures strikingly resembling »exploded metaphases» (BARBER and CALLAN, 1943). »Distributed c-mitosis», the peculiarity of which is a partition of the c-pairs in two polar groups, was exceptionally observed by MANGENOT (1942) in root-tips of *Allium Cepa* treated with colchicine; on the other hand, it seems to be the most frequent type of distribution in c-mitosis induced by methyl naphthoquinone (NYBOM and KNUTSSON, 1947). As similar c-mitosis occurred very frequently in my material, especially after the 8 hours' treatment, attention was paid to the different distribution of the c-pairs. It was noted that the most frequent distributions are: $10 + 6$, $11 + 5$, $12 + 4$; the others, $13 + 3$, $14 + 2$ and $9 + 7$, occurring more rarely. The $8 + 8$ distribution was only sometimes observed (Fig. 1 *d*). In some cases c-pairs between the two polar groups are present (Fig. 1 *e*).

According to NYBOM and KNUTSSON (1947), the so-called »pseudo-

anaphases» of WITKUS and BERGER (1944) indicate a similar phenomenon. This seems to hold true in my material, in which, especially after the 8 hours' treatment, similar pseudo-anaphases with chromosomes lacking the customary polarization and having their arms extending in all directions (WITKUS and BERGER, 1944; Microph. G, H) occur very frequently. A picture of this stage is given in Fig. 1 *f*: the chromosome fragment near one of the two polar groups has evidently arisen by a breakage in the centromeric region during the c-anaphasic splitting. A transverse division (»misdivision»; according to DARLINGTON, 1939) at the level of the centromere before and after the splitting of the c-pairs having been generally observed after the 12 hours' treatment, a comparative study of the forces acting during c-metaphase both in the 8 and 12 hours' treatments was made (for a sure interpretation of this phenomenon special attention has been paid to sectioned material). It was noted that, whereas most c-metaphases are of the »distributed» type in the first treatment, »exploded metaphases» appear to be a phenomenon of almost general occurrence in the second. As a consequence the c-pairs are typically arranged around a spherical surface in this latter case. The activity of the »achromatic sphere» being greatly increased, stretching of chromosome arms and centromere is very clear. Pictures strikingly resembling the »exploded metaphases» of BARBER and CALLAN (1943) are very easy to find in these conditions (Fig. 1 *b*, *g*—*i*). In Fig. 1 *i* and *l* two examples of misdivision during c-metaphase and c-anaphase, respectively, are figured: arrows indicate the two arms of one c-pair and of one chromosome (or two acentric fragments?) respectively.

From the observations mentioned it seems likely that the stretching of chromosomes and centromeres under the activity of the »tactoid» (NYBOM and KNUTSSON, 1947) causes the chromosomes to break down in the centromeric region. Since sticky chromosomes also break during mitosis — and not only in the case of experimental stickiness, but also in plants living under natural conditions — the examples of transverse division detected by me cannot be ascribed to the exclusive action of the drug. There is evidently a co-operation between this action and the changed mechanical properties of the chromosomes brought about by stickiness.

However, an exclusive action of colchicine seems to be responsible for the transverse division of chromosomes in root-tips of barley after two days of treatment in a 0,125 % solution of colchicine (KARPECHENKO, 1940). Also in this material it was noted that »the attachment con-

strictions of the chromosomes have become considerably longer and thinner» (l. c., p. 405). In addition to the different distributions of c-pairs described above, metaphases with c-pairs clumped in the centre of the cell were observed, together with the intermediate transitions.

When the distributing action of the above-mentioned forces is accomplished, the chromatids separate and the nucleus reverts to the resting stage. In the case of »exploded metaphases» an arrangement of split chromosomes around the »achromatic sphere», as reported by SHIMAMURA (1939), has never been detected. The observations of MANGENOT (1942), BERGER and WITKUS (1943) and NYBOM and KNUTSSON (1947), all describing a splitting of chromosomes following the accomplished activity of the »tactoid», have thus been confirmed. Fig. 1 *j*—*l*, *p* show different stages of c-anaphases and reversion process; in *k* stickiness of chromosomes — some of which already in an initial stage of reversion — is easily observed. Sometimes, while most of the c-pairs split, one or more remain undivided and undergo reversion in that condition This suggests the lack of synchronization during division of the centromeres within a cell, first observed by LEVAN (1938) and recently by STEINEGGER and LEVAN (1947) in root-tips of *Allium Cepa* treated for 24 hours in a $14000 \times 10^{-6}$ mol/l solution of *iso*-colchicine, able to induce in this condition both c-mitosis and chromosome stickiness. But, as pointed out by STEINEGGER and LEVAN (1947), splitting of chromosomes seems sometimes to be hindered by their stickiness in spite of division of the centromere having already occurred.

As a consequence of different distributions of c-pairs as well as incongruous splitting, there arise two separate nuclei, two nuclei joined by bridges, ring-shaped and very irregular nuclei some of which provided with two or more holes. Pictures quite similar to those reported for *Allium* by BERGER and WITKUS (1943) in their figures 7—16 have been observed in my experiments.

## 2. INDICATIONS OF A PRECOCIOUS REVERSION PROCESS.

The term »precocious reversion» I apply to the reversion into a resting stage (chromosome unravelling) in a period of time long before late anaphase when a partially functional spindle is still present, and before the splitting of the chromosomes during full c-mitosis. The following stages were observed:

(A) *Precocious reversion during partial c-mitosis.* — Observed at late metaphase and anaphase before complete separation of chromo-

somes both in bipolar and tripolar spindles (Fig. 1 *m*). The frequency of their occurrence seems to be very low.

(B) *Precocious reversion during full c-mitosis.* — As the most precocious reversion stage I consider the reversion of the so-called »ball-metaphases»; these are described by BARBER and CALLAN (1943) in *Triton* and have also been observed by LEVAN (1945) in *Allium* after treatment with different inorganic salt solutions. The metaphase chromosomes, more or less contracted and sometimes lying as c-pairs, seem to retain a prophase orientation and »form a closely tangled ball in the middle of the cell» (BARBER and CALLAN, 1943). A further development of these ball-metaphases seems rather unlikely, the reversion process taking place very frequently at this stage (Fig. 1 *n*). Ball-metaphase in my opinion signifies a subvital condition of the protoplasm and probably implies a poison effect. (According to BARBER and CALLAN, ball-metaphase represents a development stage following prophase—metaphase. This seems to be generally true in my material as well; but sometimes the impression can be derived that prophase—metaphases are not able to proceed further. Respecting the difficulty of a clear distinction it may be said that »ball-metaphase» can be employed as a general term in describing the different stages of one and the same process. It is in this sense that the term is employed by me.)

Reversion of c-pairs, scattered over the cell or closely clumped together (Fig. 1 *o*), is sometimes to be observed. A similar phenomenon was described by BARBER, WITKUS and SULLIVAN (1944) in *Allium*, after treatment with benzene vapour. If in these cases the centromeres have already divided, but stickiness has actually prevented the splitting of the chromatids, I cannot say with certainty.

In an attempt to explain the occurrence of these precocious reversion processes, the following two factors must be considered:

(a) *A timing factor*, to be regarded as a consequence both of a partial or total inactivation of the spindle function and of stickiness. The long duration of metaphase after treatment with colchicine has been well-known for a long time. The impeded splitting of the c-pairs in sticky chromosomes, first detected by WITKUS and BERGER (1944), has also been observed by me. In connection with these observations it is of interest to note that in pollen grains of *Tradescantia* after an X-irradiation of 360 r. the chromosomes, showing a prolonged metaphase congression and stickiness, are thicker than normal as a consequence of a superspiralization (KOLLER, 1943). But stickiness can also prolong the time required for metaphase congression and anaphasic separation

of chromosomes when a partially functional spindle is present. This prolongation caused by stickiness was clearly proved by the statistical analysis of mitotic figures in root-tips of *Vicia Faba* during the primary effect (»physiological effect», according to LEA, 1946), after neutron bombardment (NISHINA, SINOTO and SATO, 1940).

(b) *A physiological factor*, responsible for the ascertained »sticky» condition of the chromosomes, the action of which may have reference to the water metabolism of the cell. The significance of the dehydration and hydration phenomena during the chromosome cycle is, as a matter of fact, well known (see literature in KUWADA, 1939) while the vacuolization of cytoplasm during normal anaphase and in all the cells containing the various types of arrested metaphases in *Triton* (BARBER and CALLAN, 1943) is the most evident expression of a changed water metabolism. When the importance of this process for the chromosome unravelling is considered, the possibility of a precocious reversion is a very ready assumption.

## III. THE EFFECT OF ETHYLENE GLYCOL.

In the two series of experiments carried out with ethylene glycol there was no cytological difference of interest. Inasmuch as ethylene glycol S 1767 is not recommended for scientific purposes, only the material treated with the substance 1316 has been subjected to a statistical analysis in order to determine its cytological effect on sticky chromosomes by a method as appropriate as possible. In order to compare my results with those of ÖSTERGREN (1944 b), only the 4 hours', 12 hours', and 3 days' treatments will be included in this survey of my observations. The other treatments have been useful, however, for a thorough investigation of some cytological peculiarities.

The kindness of Dr. G. ÖSTERGREN in placing at my disposal all the preparations of his investigation has greatly facilitated this comparison.

### 1. 4 MOL SOLUTION.

After 4 hours the roots have not lost their turgor. A very strong liquefaction of the chromosome substance has occurred, but not to such an extent as in ÖSTERGREN's experiments. After 12 hours all roots are somewhat slack; the cytological phenomena can be compared to those described by ÖSTERGREN after the 4 hours' treatment.

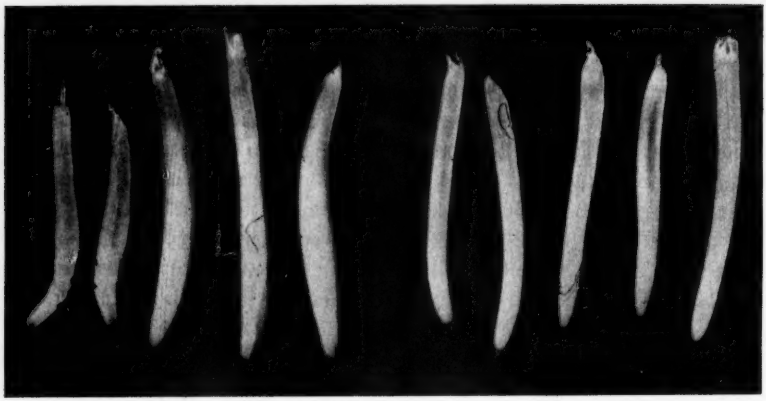
### 2. 2 MOL SOLUTION.

After 4 hours a lengthening of prophase duration appears, as is evident from the accumulation at this mitotic stage (65 %). As a consequence of a centromere inactivation (BARBER and CALLAN, 1943) many prophases, not being able to gain normal metaphasic polarization, have passed into a condition of »prophase-metaphase» and »ball-metaphase» (21,8 %). In a few cases in which the compactness of nucleus is not as strong as is normally the case in ball-metaphases, the chromosomes are scattered in the middle of the cell (Fig. 1 *t*), showing sometimes an obvious lack of matrix material and hence a very clear picture of their internal 4-partite structure.

Chromosomes of a similar type have recently been described by VAARAMA (1947) in *Allium Cepa* during partial c-mitosis induced by DDT + ethyl alcohol. Apparently centromeres are able to undergo division, chromatids »quite separate» having been observed. The splitting of the chromosomes has never been seen in my material; therefore, all these stages can be included under the general term »ball-metaphase». The course of partial c-mitosis in this treatment is typical, bi-, multipolar spindles and some c-anaphases being present; but mitoses reaching metaphase and held at this stage are rather few in number (9,2 %). The very strong chromosome contraction and the value of the Metaphase-/Ana-Telophase ratio (M/A-T = 2,30) are clear evidence of a prolonged metaphase. The degree of stickiness does not seem to be augmented as compared with the controls.

After 12 hours the percentage of prophases (19,6 %) is greatly diminished, most of them having passed into ball-metaphases (61 %), while that of metaphases is not noticeably increased (10,4 %). Since M/A-T approaches to 1, it must be inferred that after this period of time the centromeres divide in most cells. Partial c-mitosis and degree of stickiness are similar to those reported for the preceding treatment. Only in one root-tip among six studied did a greater degree of stickiness appear: peculiarly enough the percentage of »ball-metaphases» was much lower (only 44 %) and that of metaphases correspondingly greater (16,7 %), M/A-T being 1,28. Bipolar spindles being more frequent, there were also fewer spindle disturbances. This suggests an inverse relation between spindle-disturbing and stickiness-inducing activity; to a greater »sticking» activity corresponds a less spindle-disturbing one, and vice versa. A similar conclusion has also been drawn from the statistical analysis of the 1 mol treatment, the possibility of locating the two

cytological effects of ethylene glycol in different ranges of concentration thus being confirmed (ÖSTERGREN, 1944 b). In order to establish the further development of prophases and ball-metaphases, present in the 12 hours' treatment, attention was paid to the root-tips treated for 24 hours. It was noticed that a great many had reverted to the resting stage, i.e. their chromosomes showed a typical telophasic appearance; correspondingly a strong vacuolization of the meristematic cells had occurred. The final result of this process is an arrest of all mitoses and, consequently, a complete absence of mitotic figures after the 3 days' treatment, only a few reverted prophases and ball-metaphases having



*A* *B*

Fig. 3. Roots of *Allium Cepa*. — *A*: after treatment with 2 mol ethylene glycol for three days. *B*: untreated.

been counted in each root-tip. Owing to their strong vacuolization all meristematic cells have assumed a parenchymatic appearance, although their size is not greatly increased.

After three days of treatment in the 2 mol solution a c-tumour reaction was met with (Fig. 3). An experiment was then carried out with sixteen bulbs, twelve of them being treated and the others serving as controls. The results were as follows:

(a) Roots quite slack and no macroscopical effect: 2 bulbs.

(b) Lack of turgor in the meristematic region of most roots; tumours in the remaining ones: 4 bulbs.

(c) Turgor still present in all roots; tumours in most of them: 6 bulbs.

Apart from the evident poison effect, the macroscopical effect of

this treatment on the growth is a well defined one. The reaction, resulting in small and elongated tumours, is reminiscent of that recently reported by STEINEGGER and LEVAN (1947) for iso-colchicine. Parallel to the presence of tumours, lateral or symmetric breakages in the external layers of the growth region can be observed. The microscopical analysis has shown that such a breakage results from a different elongation rate in the various cell layers. When a symmetric breakage is present, this involves all the cell layers from epidermis to pericycle. It must be concluded that the destruction of the growth-polarity responsible for the typical c-tumour reaction (HAWKES, 1942) does not occur in the ethylene glycol treatment. On the contrary, the growth correlation between the various cell layers may sometimes be abolished.

In all the roots showing a macroscopical reaction turgor is present even after six days. It must be considered of interest that tumours always fail when the meristematic region of the root is slack. This seems in agreement with the observation of HAWKES (1942) that »colchicine and heteroauxin only produce a swelling behind the root-tip when that organ is still intact».

### 3. 1 MOL SOLUTION.

After 4 hours a contraction of metaphase chromosomes intermediate between that of normal and c-metaphase chromosomes, as well as a prolongation of this mitotic stage (M/A-T = 2,28), can be observed; but no spindle disturbances are still present. Some of the prophases have turned into ball-metaphases (17,3 %). More have reached metaphase (21,9 %).

After 12 hours there is a pronounced decrease in the average number of mitotic stages per root-tip ($^1/_9$ as compared with the 4 hours' treatment); there is also a great percentage (44,7 %) of cells held to metaphase (M/A-T = 2,06): partial c-mitosis being quite similar to that observed in the 2 mol treatment. In Fig. 1 *q*—*r* a multipolar spindle and a typical c-anaphase are pictured. The chromosome contraction seems to be very strong in all or most metaphases in 6 of 8 root-tips studied. In the remaining two, metaphases with normally or variously contracted chromosomes are of frequent occurrence, this being especially evident in one of them in which only 4 of 40 metaphases present a typical c-mitotic appearance.

Very interesting are the peculiar precocious reversions of prophases and ball-metaphases as well as the generalized vacuolization process in the meristematic region. A strong poison effect has thus developed, the

consequence of which is the inability of initial mitotic stages to proceed further and of at least most resting nuclei to enter mitosis. There no doubt exists a relation between increased degree of stickiness, precocious reversion and cytoplasmic vacuolization. A strong vacuolization of the meristematic cells after treatment with various chemicals is known since long back (e. g., YAMAHA, 1927). The reversion process has been especially studied by FUJII's school in experiments with living cells. It has been demonstrated that the reversion of prophase nuclei to resting stage can be induced not only by different chemicals, such as chloroform, ethyl alcohol, ether, benzene, n-butyl alcohol, $SO_2$ water, but also by pricks, plasmolysis and desiccation (WADA, 1938, 1939). As cytoplasmic vacuolization always accompanies reversion, this breakdown of mitosis has been related to the changed colloidal properties of the protoplasm (»Entmischung»; WADA, 1938, 1939). Since experiments with liquid paraffin show quite similar phenomena, there is no possibility of an intake of water during this very precocious reversion process (WADA, l. c.). I cannot state whether a similar explanation applies to my experiments. A changed protoplasmic metabolism could perhaps be inferred from the very strong blackening following osmic fixation in the root-tips in which reverted prophases and ball-metaphases are present. If the fact is taken into account that root-tips treated with a 1 mol solution maintain their turgor even after 7 or more days, it must be concluded that the above-mentioned changes in the colloidal properties of protoplasm — if they occur — are not immediately lethal.

After 3 days the average number of mitotic stages per root-tip has been reduced to a very small number (16), a few of them being reverted prophases and ball-metaphases and most of them appearing in ana- and telophase. The accumulation of these mitotic stages, which is evident from the very low value of M/A-T (0,54), is the necessary consequence of the weakened chromosome separation owing to the pronounced stickiness (cf. NISHINA, SINOTO and SATO, 1940). Metaphases similar to those described by ÖSTERGREN (1944 b), i. e. chromosomes fused into »irregularly shaped masses», have never been met with in my material. On the contrary, very long and thin chromosomes with chromatids lying widely apart and a normal equatorial disposition of centromeres appear as a rule (Fig. 1 *s*). Similar stages, also observed during my investigation on stickiness in *Allium Cepa*, have been called »prophasic metaphases» by DARLINGTON and LA COUR (1945), and their origin ascribed to »underspiralization». The very frequent fusion of sister chromatids during anaphase as a consequence of a »defective

reproduction» suggests — at least in my experiments — a »general starvation of the chromosomes» (DARLINGTON and LA COUR, 1945). As far as my present investigation and other observations on the vitality of *Allium* bulbs showing stickiness are concerned, I am inclined to include in this term both a starvation of »correctly polymerized nucleic acid» and a diminished production of the basic proteins contained in the chromosomes (CASPERSSON, 1936).

Underspiralization of chromosomes in the 3 days' treatment is easy to understand in view of the fact that all mitotic stages in this case involve nuclei which passed through the initial action of the 1 mol solution in the resting stage and entered mitosis in a period of time in which the strong »sticking» activity of the compound had completely developed.

### 4. 1/2 MOL SOLUTION.

There is a greater chromosome contraction than normal, parallel to a protracted duration of the metaphase (M/A-T = 1,80). Evidently these phenomena depend on an initial shock action. The percentage of ball-metaphase is very low (5,9 %), as compared with the corresponding pictures in 1 mol solution.

After 12 hours the average number of mitotic figures has been reduced to about 50 %, all metaphases and subsequent mitotic stages having reassumed their normal course (M/A-T = 0,99).

After 3 days the statistical analysis shows a small increase in the number of mitotic stages; this could suggest an inurement of the cells to the compound. This being true, the two different percentages of prophases in the 12 hours' (45,4 %) and 3 days' (50 %) treatments respectively afford confirmatory evidence.

In Table 1 the statistical data and the most important observations concerning the cytological effect of ethylene glycol on sticky chromosomes have been gathered. The average number and percentage of mitotic stages were established by counting all stages present in 3 root-tips for each treatment, the 4 mol solution excluded. Since some cells may be lost during the dehydration process previous to inclusion in Canada balsam, the counts made do not represent the number of mitotic stages actually present in the root-tip. However, as a similar error is possible in all cases and the counts generally comprise a very great number of stages, the percentages obtained should be fairly accurate. On the other hand, an attempt to count mitotic stages in sections has proved to be much less successful. The following general

TABLE 1. *The cytological effect of ethylene glycol on sticky chromosomes in Allium Cepa.*

+ = presence and — = absence of the effect in question; ++, +++, ——— = different degrees of intensity, plus or minus, of the effect in question.

| Mol. concentr. | Exposure | Average number of mitotic stages per root-tip | Percentage of mitotic stages | | | | Partial c-mitosis | Chromosome contraction | Metaph. / Ana-teloph. Ratio | Stickiness | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Proph. | Ball-metaph. | Metaph. | Ana-teloph. | | | | | |
| Not treated | — | 804 | 53,1 | 0,2 | 23,0 | 23,7 | — | — | 0,97 | + | |
| 2 mol | 4 hours | 785 | 65,0 | 21,8 | 9,2 | 4,0 | + | +++ | 2,30 | + | Bi-, multi-polar spindles and typical c-anaphases |
| | 12 hours | 791 | 19,6 | 61,0 | 10,4 | 9,0 | + | +++ | 1,15 | + | Bi-, multi-polar spindles and typical c-anaphases |
| | 3 days | 5—6 | — | — | — | — | — | — | | — | |
| 1 mol | 4 hours | 772 | 51,2 | 17,3 | 21,9 | 9,6 | — | ++ | 2,28 | + | No spindle disturbances |
| | 12 hours | 96 | 13,0 | 20,6 | 44,7 | 21,7 | + | +++[1] | 2,06 | ++ | Bi-, multi-polar spindles and typical c-anaphases<br>Many prophases and ball-metaphases of »reverted» type |
| | 3 days | 16 | 7,3 | 5,8 | 30,6 | 56,3 | — | ——— | 0,54 | +++ | Prophases and ball-metaphases all reverted |
| 1/2 mol | 4 hours | 866 | 49,5 | 5,9 | 28,6 | 16,0 | — | + | 1,80 | + | |
| | 12 hours | 387 | 45,4 | 1,7 | 26,3 | 26,6 | — | — | 0,99 | + | |
| | 3 days | 416 | 50,0 | 0,6 | 24,8 | 24,6 | — | — | 1,00 | ++ | |

[1] Generally very strong. See description in the text.

conclusions can be drawn as to the cytological effect of ethylene glycol in my material:

(a) An increased tendency to chromosome contraction and prolongation of metaphase stage in all the initial treatments, as well as to spindle disturbances (partial c-mitosis also after 12 hours in the 1 mol solution);

(b) A common inactivation of the centromeres, resulting in the frequent occurrence of ball-metaphases, especially in the 2 and 1 mol treatments;

(c) A physiological inability of the cells to reach metaphase, precocious reversion of prophases and ball-metaphases and strong vacuolization of meristematic cells appearing as a rule after treatment with 2 mol (24 hours) and 1 mol (12 hours) solutions;

(d) Probably a greater resistance to superficial changes in the colloidal properties of the substances constituting chromosome matrix.

## IV. DISCUSSION.

That the stickiness signifies changed physiological conditions within the cell is beyond all doubts, although its causes, with our present knowledge, cannot be explained with certainty. Stickiness is the consequence of one and the same basic physiological phenomenon, whatever the causes or the means, experimentally controllable, as regards its induction.

That dormant bulbs of *Allium Cepa* exposed for some time to low temperatures show stickiness does not seem astonishing in view of the great water content of these organs and consequently the high rate of metabolic processes also during dormancy. Stickiness in these bulbs being generally confined to adhesion phenomena, and the fusion of sister chromatids and fragmentation appearing only in exceptional cases, this stickiness may be compared with that reported by earlier authors for root mitosis of plants living under natural conditions (e. g., RESENDE, 1941 and PINTO-LOPES, 1946) and assumed to be — although not generally — a vital phenomenon. The above-mentioned observations on the vitality of bulbs exposed to low temperatures and other experimental conditions to be referred to elsewhere may very well indicate a different reaction of sticky chromosomes to the same agent as compared with normal ones. The present results on the cytological effect of ethylene glycol seem to be evidence in the same direction, when account is taken of the fact that ÖSTERGREN's experiments, carried

out during the summer, certainly involved bulbs in different physiological conditions.

As to the effect of colchicine in my material, nothing can be said with certainty. Observations of interest relate not only to the morphological appearance of the chromosomes during c-mitosis, but also to »misdivision», i. e. breakage in the centromeric region, and to different stages of precocious reversion. Moreover, »distributed c-mitosis» and »exploded metaphases» have appeared to be an almost general phenomenon during c-metaphase.

Similar distributions of chromosomes during c-mitosis do not seem to be of frequent occurrence, most cases having been reported in a condition of stickiness both in plants (WITKUS and BERGER, 1944; NYBOM and KNUTSSON, 1947) and animals (BARBER and CALLAN, 1943, p. 264). But pictures strikingly resembling my own were obtained by BERGER and WITKUS (1943) in *Allium Cepa* after treatment with a 0,25 % solution of colchicine. The concentration of the drug having no influence on the course of c-mitosis — quite similar phenomena have been observed in root-tips treated with a 0,25 % solution —, the only difference between my results and those of the American authors is that my investigation started with a condition of ascertained stickiness. BERGER and WITKUS (1943) did not report having met with stickiness in their experiments. The peculiar appearance of some c-pairs and the distinct lack of matrix shown by the chromosomes, already pointed out by VAARAMA (1947), are in my opinion evidence of sticky chromosomes. It may be that the above-mentioned writers attached no importance to the stickiness — perhaps already present in the untreated root-tips or induced by impurities in the colchicine solution.

## SUMMARY.

A study was carried out on the effect of colchicine and ethylene glycol in root-tips of *Allium Cepa* showing stickiness (bulbs coming from a storehouse in which minimal temperatures ranging from 0° to — 4° C. were recorded). In the colchicine experiments, peculiar appearances of c-mitosis owing to stickiness within and between chromosomes, »misdivision» of chromosomes, »distributed c-mitois» and »exploded metaphases», i. e. arrangement of all c-pairs around a spherical surface, were observed; as well as many precocious reversion processes, owing to the peculiar physiological conditions in which c-action had developed.

In the ethylene glycol experiments the following phenomena were observed in addition to previous observations (ÖSTERGREN, 1944 b):

(a) A general lengthening of metaphase duration in all the initial treatments and partial c-mitosis also after treatment in an 1 mol solution for 12 hours;

(b) A wide tendency of prophases to pass into ball-metaphase especially after the 2 mol and 1 mol treatments;

(c) A precocious reversion of prophases and ball-metaphases after treatment with 1 mol (12 hours) and 2 mol (24 hours) solutions and a simultaneous vacuolization of meristematic cells, this phenomenon being interpreted as evidence of a poison effect;

(d) Probably a slightly greater resistance to surface changes in the colloidal properties of matrix.

In discussing the results attention is claimed to the ascertained condition of stickiness in which the experiments were started.

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# THE CONCEPT AND MECHANICS OF CHROMOSOME REDUCTION

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BY chromosome reduction we mean that process, taking place during the so-called maturation divisions, the last two mitoses in that series of cell divisions leading to the production of sex cells or — as usually in plants — spores, as a result of which the gametes, or (in plants) already the gametophyte from which they arise, receive only half of the number of chromosomes characteristic of the other cells of the species.

The mechanism of reduction is based, with some exceptions, on the formation of bivalents. Since a bivalent is formed by the union of a paternal and the corresponding maternal chromosome, the part played by the maturation divisions with respect to reduction was during the earliest stages of cytological research *a priori* conceived to be the following: One of these divisions separates the paternal from the maternal part of the bivalent, or, in other words, separates the original homologous chromosomes from one another; the other division splits each original chromosome in the manner of a normal mitosis. The former type of division is called reduction and the latter equation division, and the question as to which of the maturation divisions is to be considered reductional and which equational has been much discussed in the early literature on cytology.

This question was rather unexpectedly and in some respects finally solved when the nature of the chiasmata and therewith the real structure of the bivalents was finally cleared up in the early 1930'ies, chiefly through the efforts of DARLINGTON. It appeared that the chiasmata cause two chromosomes differing in their genetic constitution from the original paired chromosomes to arise, each of which new chromosomes thus contains matter of both paternal and maternal origin in different proportions, depending on the number and original location of the chiasmata. Since the same chromatid may contain matter of both paternal and maternal origin, it is clear that these two kinds of matter cannot be entirely separated from one another in the maturation divisions. With respect to each separate gene, as well as with respect

to the portion of a chromosome situated between two chiasmata, one or the other of the maturation divisions is always reductional and thus brings about alleles segregation, while the other division is equational and separates identical sister genes from one another. But when the bivalents are considered as a whole the question as to reduction or equation division is meaningless.

*Even though the question of reduction may thus be considered to have lost its genetical importance, the morphological problem still exists.* It must, in fact, be pointed out that a bivalent is not only made up of four chromatids but, more particularly, of two chromosomes, one of which is composed of the two chromatids with a common paternal centromere and the other of those with a common maternal centromere. For this reason a bivalent may be considered divisible into its four components during two maturation divisions in two principally different ways. The first possibility is that the two chromosomes composing the bivalent and, more particularly, their centromeres separate from one another in the first division and divide in the second; the other possibility is that both chromosomes and their centromeres divide in the first maturation division, while the chromatids and daughter centromeres thus arising are separated from their homologues in the second division. Regarding the matter thus in the morphological sense, the reduction division is naturally the one that separates the chromosomes of a bivalent from one another, for this is the division that reduces the chromosomes to one half of their original number. Equation division is then, correspondingly, the division in which the chromosomes are divided, for in this the number of chromosomes undergoes no change. In the morphological sense — but only thus — we are then justified in bringing up again the question as to whether the first or the second maturation division is to be considered the reduction division; do we have pre- or post-reduction? And — it must be remarked — this is just the sense, that is to say as a morphological problem, in which the whole question of reduction has really always been investigated. The attempts to clear up this problem have been undertaken on the basis of morphological clues, even during the period when the question was thought to be of genetical importance also. For this reason even the earliest investigations in the field still deserve notice, in principle at least, in a chromosome morphological consideration of the problem of reduction.

Inspection of the literature pertinent to this problem (e.g., GREGOIRE, 1905, 1910; REUTER, 1930) shows us that the first maturation division

has always and since the beginning been most generally held to be the reduction division. Characteristic particularly of the early period of cytological research is the concept that all the chromosomes of the same cell undergo reduction in the same division.

Particularly this last-mentioned concept, however, soon attracted critical attention from those investigators who had found heteromorphic chromosome pairs in their research objects — usually grasshoppers. For they were able to demonstrate that the heteromorphic portions of such bivalents behave differently during the maturation divisions in different cases, and from this finding they drew the conclusion that some of the chromosomes in one and the same cell may be subject to pre- and others to post-reduction as well as that the same bivalent may sometimes divide pre- and sometimes post-reductionally. The question is presented in this light even in some well-known handbooks and textbooks (e. g., BELAR, 1928).

More modern cytology (as, for instance, DARLINGTON, 1932, 1939) has, however, taken a diverging stand on this question. According to its teachings the phenomenon of reduction is everywhere strictly uniform, and takes place more particularly in such a manner that the first maturation division is always the reduction division. No other alternative could be possible, if for no other reason then only because the centromere is, according to DARLINGTON, not yet capable of division on coming into the metaphase of the first maturation division. On these grounds DARLINGTON explains the behaviour of the heteromorphic chromosomes during the maturation divisions as depending on whether a chiasma arises between the centromere and the heteromorphic portion of the chromosome or not. If the interval between the centromere and the heteromorphic portion is so short that no chiasma ever forms there, then the chiasma forms on the other side of the centromere, and thus in the first maturation division the heteromorphic portion follows in its entirety the centromere of the chromosome to which it originally belonged. The division is then reductional with respect both to the heteromorphic portion and to the whole bivalent. If, on the other hand, one chiasma always appears between the centromere and the heteromorphic portion of the chromosome, then the latter always divides already in the first maturation division. In this case the first division is equational with respect to the heteromorphic portion, but nevertheless a reduction division with respect to the whole bivalent. This is true because the chromatids of the heteromorphic portion really already belong to different chromosomes as soon as the chiasma arises, and just

for this reason they are separated already in the first maturation division. Thus, the equational division of the heteromorphic portion does not justify considering the division as equational also with respect to the entire bivalent, as was formerly the practice. It is further possible for the interval between the centromere and the heteromorphic portion to be of such a length that a chiasma may or may not arise there, or that the number of chiasmata varies. In consequence, the heteromorphic portion may in this case divide either in the first or in the second maturation division, but with respect to the bivalent as a whole the division is even then always reductional, inasmuch as the ultimate criterion is always the fate of the centromere.

In this light we are able also to explain the behaviour of the type of bivalent observed, for example, in *Mecostethus* (e. g., WHITE, 1936). Here the chiasma usually arises on the long arm of the acrocentric chromosome, in the immediate neighbourhood of the centromere; nor does it move thereafter. In consequence, the main portions of both original chromosomes divide equationally in the first maturation division. In reality the case is, nevertheless, one of pre-reduction, for the centromeres and the new chromosomes arising when the chiasma forms do not divide but separate from one another (Fig. 1).

But even if this has been fully understood a final solution of the question of reduction has not been reached, for of late it has been possible to demonstrate the fact *that real post-reduction also occurs, in which the first maturation division does not separate entire chromosomes and centromeres from one another but actually divides them.* (We do not refer here to the many insect sex chromosomes and autosome univalents as found in many plants which already divide equationally in the first meiotic division.)

This is the state of affairs the present author has shown to exist in dragon-flies (OKSALA, 1943). The bivalents of the male odonate are generally normal rod bivalents, in which the single chiasma is completely terminalized. The bivalents take a position on the metaphase plate with their longer axis perpendicular to the axis of the spindle in the first maturation division. This indicates that each of the two centromeres is independently oriented with respect to the poles, and that they already divide in the first maturation division. Under favourable circumstances it is also possible to observe two separate spindle fibres attached to each bivalent, corroborating the diagnosis of centromere auto-orientation. Some of these species (e. g., *Aeschna grandis*) also possess a heteromorphic chromosome pair, the heteromorphic portion

of which already divides in the first maturation division even though no chiasma ever forms between it and the centromere. This fact natur-

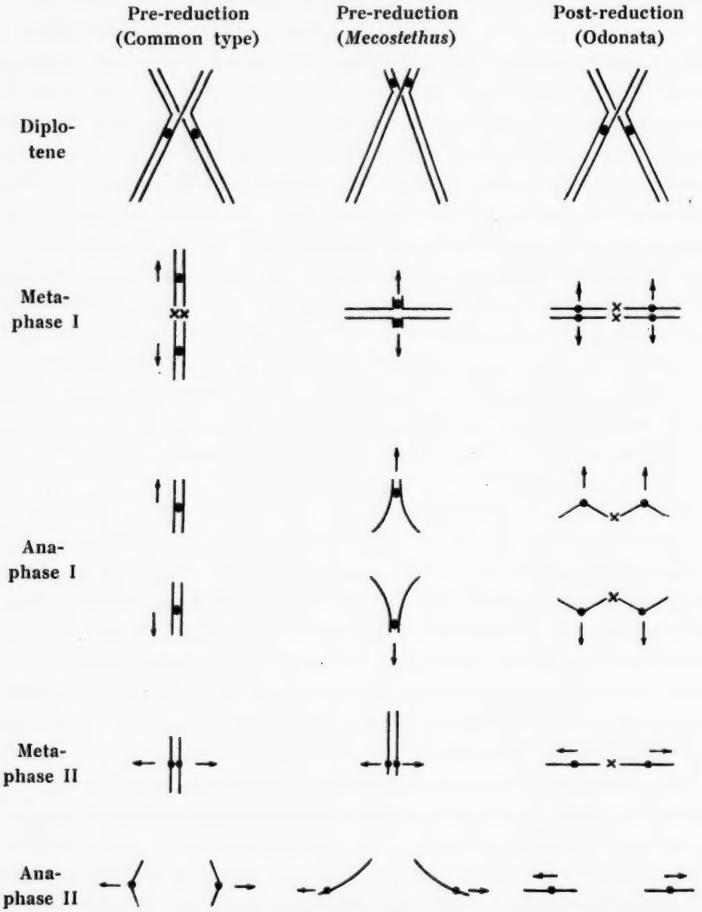


Fig. 1. A scheme of the two possibilities of the chromosome reduction. Pre-reduction is represented by two different bivalent types. — ⨯⨯ = terminal chiasma, ⨯ = half-chiasma.

ally gives particularly conclusive proof that the case is one of post-reduction.

The present author has also shown post-reduction to take place in the female odonates (OKSALA, 1945). In the female, however, two

chiasmata per bivalent arise, terminalizing toward opposite ends and thus forming a ring. These rings take up a position in the plane of the metaphase plate, a natural result of centromere auto-orientation.

All the essential differences between pre- and post-reduction may be seen from the accompanying diagram (Fig. 1).

On the basis of this diagram we may now proceed to clear up a number of concepts. In the first place it may be pertinent to remark that in the case of pre-reduction the halves of the bivalents separating from one another in the first maturation division are real chromosomes with a centromere common to both chromatids; whereas in the case of post-reduction these halves are not actually chromosomes, to be precise, inasmuch as each of them is composed of two chromatids deriving from different chromosomes, the centromeres also being only homologues but not sister centromeres. The most suitable name for such structures would seem to be *double chromatid.* »Dyad» would be a good name, too, but since this has also been used as a synonym for tetrad (bivalent) — Ris (1942, p. 293) moreover applies the same designation, dyad, to each of the two chromosomes of the same bivalent —, it would seem impossible to use that term in this new sense.

The fate of the chiasmata in each of these two types of reduction is also different. In the pre-reductional type the first maturation division breaks the chiasmata, whereas in the post-reductional type the chiasmata are in a certain sense preserved up to the second maturation division. Since these structures, however, no longer correspond to the definition of a chiasma, »a point in which each of the four chromatids of a bivalent changes partners», the present writer designates them as *half-chiasmata,* thus indicating these structures to have arisen out of the division into two parts of the original chiasmata.

Now, considering the possibility of both pre- and post-reduction, we may correspondingly define reductional and equational division as follows: *Reduction division is that maturation division in which either the whole chromosomes* (*pre-reduction*) *or chromatids deriving from different chromosomes* (*post-reduction*) *separate from one another; equation division, on the other hand, is that maturation division in which the chromosomes divide, whether they be chromosomes already subjected to reduction* (*post-equation*) *or not* (*pre-equation*).

Outside of the odonates post-reduction occurs also in the coccids and the aphids, as Ris (1942) has shown. Since, however, these insects have so-called diffuse centromeres it is impossible to take the behaviour of the centromeres during the maturation divisions as a criterion of the

division types in this case, as we can in those forms with a distinct centromere. Inasmuch, however, as the terminalization of the chiasmata is complete, and both chromosomes of the bivalents for this reason clearly distinguishable from one another, post-reduction is so evident an observable fact that there is no doubt of its occurrence even in these species.

The problem of reduction might become critical and even in principle impossible of decision if cases were found in which the centromeres were diffuse and in which the chiasma appeared in the middle of the chromosome not finally terminalized toward the end. In such a case it would be impossible to decide, not only in practice but even in principle, which of the four chromatids of a bivalent belonged to any one chromosome; there would be no sense in bringing up the question of reduction at all. Still, we must take into consideration not only the fact that no such case has yet actually been observed but also that such a condition is cell-mechanically evidently impossible and thus cannot exist at all, at least not continuously. For, since in lack of a distinct centromere the chromosome is everywhere equally capable of polar orientation, each arm of such a cross-shaped bivalent would tend independently to orientate itself with respect to the poles. As a result of this situation different portions of the same chromatid might be attracted toward different poles during the anaphase, which would naturally lead to serious disturbances in the course of division. In the light of this ratiocination it is more than interesting to record the fact that there are never any but completely terminalized chiasmata to be observed in the aphids and coccids.

We may therefore, in my opinion, assume that no case exists or can exist in which a solution of the problem of reduction is not possible at least in principle.

Whether the phenomenon of post-reduction is limited only to the odonates, aphids, and coccids, or whether it may possibly occur even in other groups is as yet unknown. It is possible that thorough investigation may yet disclose the occurrence of post-reduction also elsewhere, but this type of reduction will in any case remain rare, for a very large number of groups have already been shown to be clear cases of pre-reduction.

What, then, is the reason for pre- or post-reductional division of the chromosomes? Of course, the reduction type depends directly on whether or not the chromosomes and particularly their centromeres are already capable of division on entering the metaphase of the first

maturation division. If they are, the result is post-reduction, which is thus comparable to an ordinary mitosis and as such to be considered the more primitive type. If not, then pre-reduction is the sole remaining possibility. What, on the other hand, determines the capability or lack thereof of the chromosomes and centromeres to divide in the first maturation division, thus indirectly also deciding the reduction type, is a problem that leads us to the question as to the nature of the entire meiotic system, a question we must refrain from touching on in this limited exposition.

## SUMMARY.

Though the question of the pre- or post-reductional nature of the division of the bivalents must be considered to have lost its genetical importance, the problem still exists in a morphological sense. If a bivalent divides in the first maturation division in such a manner that both the whole chromosomes of which it is composed, and the homologous centromeres, separate from each other (common type), a pre-reduction is in question. If, on the other hand, the division takes place so, that the chromosomes and centromeres actually divide in the first maturation division (dragon-flies, aphids, coccids), we have a post-reduction. In the case of post-reduction the »chromosomes» in the second maturation division are not ordinary chromosomes, since they contain both a paternal and a maternal centromere, but *double chromatids*, the halves of which are connected by a *half-chiasma* resulting from the division of the original terminal chiasma.

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# FURTHER STUDIES IN SPONTANEOUS AND SYNTHETIC CALAMAGROSTIS PURPUREA

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IN Scandinavia there are two *Calamagrostis* species belonging to the group Homeotricha TORGES, viz. *C. canescens* (WEBER) ROTH (= *C. lanceolata* ROTH) and *C. purpurea* (TRIN.) TRIN. The two species are very closely related. The former, however, is an amphimict with 2n = 28, the latter an apomict with chromosome numbers varying between 56 and 91 (NYGREN, 1946, p. 202). *C. purpurea* extremely seldom develops pollen, its anthers are I-shaped, yellow and do not dehisce. In the loculi the P.M.C's generally lie degenerated in big plasmodia. *C. canescens*, on the other hand, has very good pollen, its anthers being X-shaped, purple and dehiscent. It constitutes a sexual allogamous species and does not give any seeds after bagging, while *C. purpurea* gives abundant seeds. The two species are usually separated in the floristic handbooks by the hairiness of the laminae, the length of the ligula and the number of nodes on the straw. Thus *C. canescens* should have hairy laminae, a 3 mm long ligula and only 4—5 nodes, while the characters of *C. purpurea* are smooth laminae, a 10 mm long ligula and 6—8 nodes (LEHBERT, 1918, p. 18). After careful studies for many years the author, however, could show that the length of the ligula and the number of nodes vary greatly in *C. purpurea* plants of different age, at the same time as the hairiness could change from one year to another in the same clone plant (NYGREN, 1946, p. 201). Thus a *C. canescens* plant had smooth laminae in three of four seriate years, and a *C. purpurea* clone showed hairy laminae in 1944 and 1947, but had smooth laminae in 1943, 1945 and 1946.

Some *C. purpurea* clones look completely like *C. canescens*, others resemble hybrids between *C. canescens* and the amphimictic species *C. arundinacea*, *epigeios* and *neglecta*. In all cases studied these hybrids could be separated from *C. purpurea* by the fact that they had pollen in every loculus. The rare *C. purpurea* plants developing pollen do this only in some panicles or in solitary loculi.

*C. purpurea* plants with high chromosome numbers in most cases form hybrids with amphimictic species. They generally have the chromosome number 70 in somatic plates, having arisen by fusion of an unreduced gamete from *C. purpurea* with a reduced one from an amphimictic species ($56 + 14 = 70$). Sometimes the numbers 63, 77, 84 and 91 occur. The number 63 indicates the co-operation of a reduced female gamete, while the three high numbers can be easily explained if pollen from *C. purpurea* itself has taken part in forming the zygotes.

As many things indicated that *C. purpurea* had arisen from *C. canescens*, or from original forms and hybrids of this species, the present author six years ago planned to synthesize the species by systematic crossings. I began to cross *C. canescens* and *C. epigeios*, as hybrids between these species in Nature resembled *C. purpurea* in many respects and, in addition to this, the distribution of the two amphimicts corresponds very well with that of the apomictic species. As the chance of obtaining hybrid plants with the chromosome number 56 was very slight if the parents both had $2n = 28$, I used a *C. epigeios* plant with 42 chromosomes somatically. Such plants can be reared by hybridizing *C. epigeios* with $2n = 28$ and 56. The *C. epigeios* plant was used as mother on account of its capacity to form unreduced gametes. Three different *C. canescens* biotypes were used. In 1942 no hybrids were obtained, but when, in 1943, the crossings were made on a large scale seven hybrid plants were obtained in a total of three crosses. Two belonged to the same combination. The seven plants all flowered in the summer of 1946 (one flowered in 1945). The plants looked completely like some *C. purpurea* clones from Nature.

In 1944 seedlings from *C. canescens* were treated with colchicine (NYGREN, 1946, p. 211). Twelve plants with the double chromosome number were obtained, all having $2n = 56$. The plants flowered in the summer of 1946 and it could now be confirmed that they belonged to the species of *C. purpurea* and not to that of *C. canescens*.

## I. CALAMAGROSTIS EPIGEIOS ($2n = 42$) × CALAMAGROSTIS CANESCENS ($2n = 28$) = C. PURPUREA ($2n = 56$).

(Figs. 6 and 18 respectively; 3 and 15 respectively; 7 and 16 respectively; 8 and 9.)

Two of the three successful crossings belonged to the same combination (Ly 2—38 × J 6—38), while in the third use was made of a *C. canescens* plant from Eskilstuna (3—41 A).

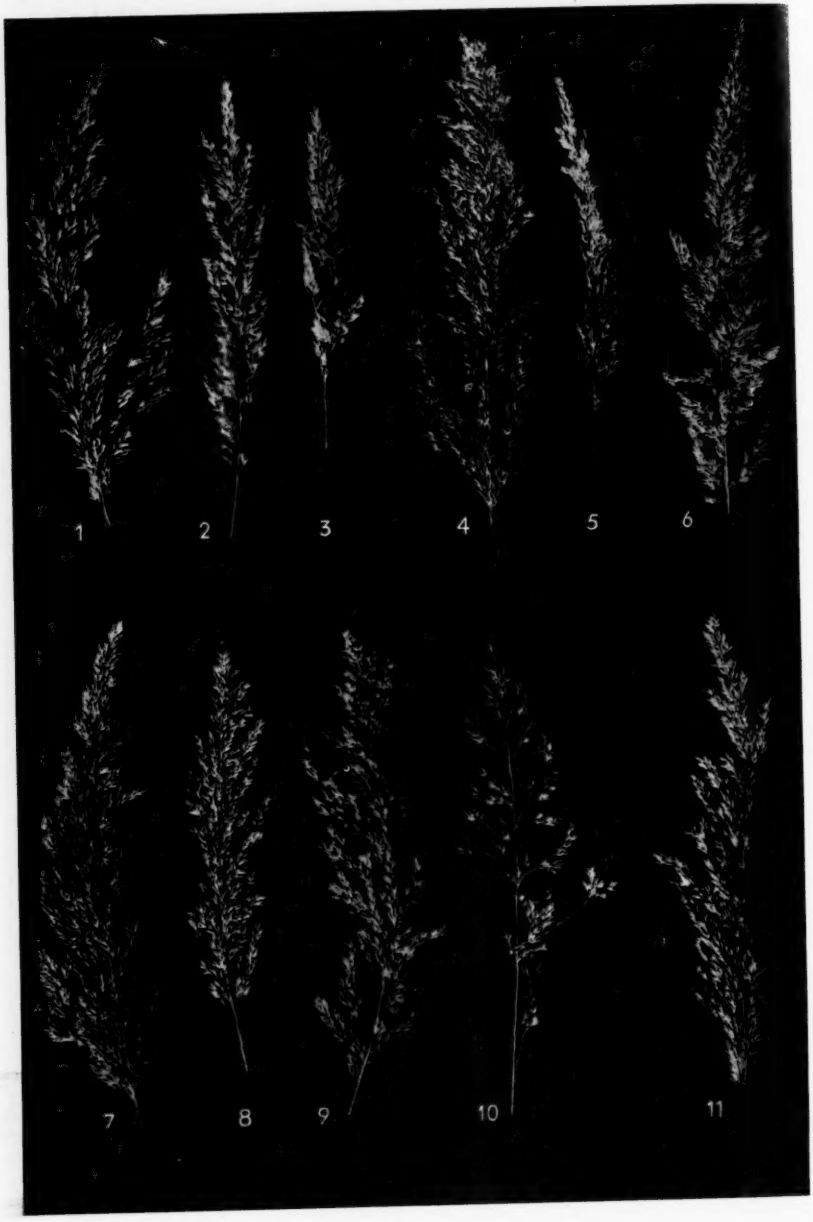
In the summer of 1946 measurements were made on parent plants and hybrids. In all cases the glumes of 20 spikelets from different parts of one panicle were measured on each plant. The length of the ligula was examined on the uppermost lamina of every straw on the plants. The hairiness of three laminae on all straws was tested. No variation could be found on the same plant in this respect. However, it was an interesting fact that the *C. canescens* plant J 6—38 from Östersund was hairy in 1945, while in 1946, just as in 1944 as well as in 1947, it was smooth. The length of the ligulae was found to vary very little, probably because, on every type, all straws examined belonged to the same plant and measurements were made on leaves with the same location on the straw. The length of the glumes varied, however, as is clear from Table 1. *C. epigeios* (Fig. 18) has somewhat longer glumes than *C. canescens* (Fig. 15), often drawn out into a subulate point. In addition they have short, coarse bristles. In all hybrid plants the glumes were somewhat broader near the base than in either parent. Their form resembled *C. canescens*, but they showed the hispidity of *C. epigeios*. In the parents, as well as in the hybrids, the callus hairs were somewhat longer than the lemmas. The branches of the panicles in the hybrids were characterized by just the same short, coarse bristles as in *C. epigeios*.

TABLE 1. *Synthetic Calamagrostis purpurea in relation to its parents, Calamagrostis epigeios (mother) and C. canescens (father).*

| Plant No. | Species | Leaf hairs | Length of ligula | Length of glumes 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | M (mm) | Relation lemma/callus hairs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J 6—38 | *C. c.* | +— | 2,0 | | | 17 | 3 | | | | | | 4,22 | Hairs longer |
| E 3—41 | *C. c.* | 0 | 3,8 | 15 | 3 | 2 | | | | | | | 3,44 | » » |
| Ly 2—38 | *C. ep.* | 0 | 13,0 | | | | | | 5 | 14 | 1 | | 6,44 | » » |
| 43—44 | *C. p.* | 0 | 12,0 | | 2 | 17 | 1 | | | | | | 4,06 | » » |
| 49—44 | *C. p.* | 0 | 7,1 | | | 3 | 15 | 2 | | | | | 4,56 | » » |
| 3— 5 | *C. p.* | 0 | 11,6 | | | 16 | 4 | | | | | | 4,35 | » » |
| 3—12 | *C. p.* | 0 | 7,0 | | | 20 | | | | | | | 4,16 | » » |
| 3—13 | *C. p.* | 0 | 13,2 | | | 6 | 12 | 2 | | | | | 4,58 | » » |
| 3—14 | *C. p.* | 0 | 10,0 | | | 5 | 4 | 11 | | | | | 4,93 | » » |
| 3—15 | *C. p.* | 0 | 8,7 | | 1 | 17 | 2 | | | | | | 4,29 | » » |

Length of ligula in mm, of glumes in tenths of a mm. The father plant J 6—38 is hairy or smooth in different years. — 43—44 has been raised by crossing E 3—41 and Ly 2—38, in the remaining cases J 6—38 is the father plant. — *C. c.* = *Calamagrostis canescens*; *C. ep.* = *Calamagrostis epigeios*; *C. p.* = *Calamagrostis purpurea.*

The cytological investigation revealed that the hybrids, without exception, lacked the capacity of developing meiosis on the male side. All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P.M.C's degenerated sooner or later, just as the situation is in *C. purpurea* in Nature (cf. NYGREN, 1946, p. 156). No pollen was formed, the P.M.C's constituting plasmodia. The same phenomenon occurs, at least in most loculi, in a hybrid between *C. canescens* and *C. epigeios* from a natural locality ($2n = 28$) and in a synthesized hybrid between *C. arundinacea* and *epigeios* ($2n = 42$; cf. NYGREN, 1946, pp. 239—240). My studies of the above-mentioned *C. arundinacea* hybrid have already shown that the mitotic type of division in the E.M.C's, characteristic of many apomicts, can be developed in a hybrid between two amphimicts; cf. NYGREN, l. c. This has now been confirmed also on this material, though the division itself has not yet been found. However, resting stages are quite enough to determine this type of division. The *C. arundinacea* hybrid did not give any seeds after bagging, at least no seedlings were developed, but the plant showed a high percentage of unreduced gametes after free flowering. The synthetic *C. purpurea* plants have all given seeds after bagging, the germination percentage, however, is very low, only 2—3 seedlings could be raised in each number. The seeds were unfortunately not sown out before the beginning of June, 1947; thus the seedlings in the autumn of this year are too small for a determination of the chromosome numbers. This circumstance, however, is not very serious, as the hybrid plants were bagged and lacked the capacity of forming pollen. Besides this, the cytological studies showed that in the E.M.C's the mitotical division occurs, which involves a continued development of the E.M.C. according to the diplosporous *Antennaria* scheme (cf. GUSTAFSSON, 1946). For these reasons the synthetic *C. purpurea* plants must be regarded as apomicts. Perhaps the small number of seedlings depends on the plants being only capable of developing some egg-cells autonomously. If this is the case here, though obviously not in the above-mentioned *C. arundinacea* hybrid, it may be due to the higher chromosome number in the synthetic *C. purpurea* plants (cf. HÅKANSSON, 1943 and 1944).

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Figs. 1—11. Panicles. — 1. Gäll. 1—41 (*C. p.* $2n = 56$). — 2. Haploid Gäll. 1—41 ($2n = 28$). — 3. J 6—38 (*C. c.* $2n = 28$). — 4. Colchicine-treated J 6—38 ($2n = 56$, synthetic *C. p.*), plant 7. — 5. As No. 4, plant 1. — 6. Ly 2—38 (*C. ep.* $2n = 42$). — 7. 3—5 (synthetic *C. p.*, $2n = 56$, raised in a cross between J 6—38 and Ly 2—38). — 8. 43—44 [synthetic *C. p.*, $2n = 56$, raised in a cross between Eskilstuna 3—41 (*C. c.* $2n = 28$) and Ly 2—38]. — 9. As No. 7, another cross. — 10. 17—21 (*C. ep.* mutation in a *C. p.* clone. $2n = 56$). — 11. 17—3, another plant of the same clone as the mutation No. 10. $2n = 56$. — $6 = 1/4$ of nat. size, 1—5, 7—11 $= 1/2$. — (Photo of Figs. 1—18 by C. G. ALM.)

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## II. CALAMAGROSTIS PURPUREA (2n = 56) RAISED BY COLCHICINE TREATMENT OF SEEDLINGS FROM CALAMAGROSTIS CANESCENS (2n = 28).

(Figs. 3 and 15 respectively; 4 and 17 respectively; 5.)

It is generally difficult to obtain seeds in Nature of *C. canescens*, because the species is allogamous and propagates by stolons to a large extent. Thus, in large areas one and the same clone may be the only one occurring, a circumstance which involves the complete absence of seed formation. In my cultures of *Calamagrostis*, however, many biotypes of *C. canescens* stand beside one another, and for that reason the seed-setting is good. — Many thousands of seeds from the above-mentioned *C. canescens* plant, J 6—38, were treated with colchicine in the spring of 1944. The seedlings were put in soil-boxes, and only the most affected were preserved. The chromosome numbers of 15 plants that were obviously influenced by the treatment were determined. Plates with 56 chromosomes could be counted in 12 of these, the other three plants had 2n = 42. Only the first-mentioned plants survived. As early as in the summer of 1945 I could confirm that the treated plants belonged to the species of *C. purpurea* (see NYGREN, 1946, p. 211). The nerves of the leaves were broader than in J 6—38, and the stomata bigger and agreeing with the size in *C. purpurea* from Nature. A peculiar circumstance was that the leaves were smooth, while the »mother» plant in the summer of 1945 had hairy laminae. As J 6—38 had no hairs in 1946 as well as in 1947, the phenomenon can be explained by assuming that the development of hairs in these types was due to external conditions (cf. GUSTAFSSON, 1947, p. 113). The values for these plants are given in Table 2.

The length of the ligula in the colchicine-doubled plants deviates very strikingly from that of J 6—38 and corresponds to the actual conditions in spontaneous *C. purpurea*. The glume length is about the same, but the glumes are more covered with short bristles in the doubled plants. Such short bristles are characteristic of *C. purpurea* (cf. Figs. 17 and 12). It is an important fact that haploid *C. purpurea* (Fig. 2) shows the same relation to the mother plant regarding these bristles as J 6—38 before and after doubling (Figs. 12 and 13).

Figs. 12—18. Spikelets. — 12. Gäll. 1—41 (*C. p.* 2n = 56). — 13. Haploid Gäll. 1—41 (2n = 28). — 14. 17—21. *ep.* mutation in a *C. p.* clone. 2n = 56. — 15. J 6—38 (*C. c.* 2n = 28). — 16. 3—5, synthetic *C. p.* raised by crossing J 6—38 and Ly 2—38 (2n = 56). — 17. J 6—38, synthetic *C. p.*, raised by colchicine treatment (2n = 56). — 18. Ly 2—38 (*C. ep.* 2n = 42). — About 4 ×.

TABLE 2. *Synthetic Calamagrostis purpurea, raised by colchicine treatment of seedlings from Calamagrostis canescens, in relation to the original plant.*

| Plant No. | Species | Leaf hairs | Length of ligula | Length of glumes 39 | 42 | 45 | 48 | M (mm) | Relation lemma/callus hairs |
|---|---|---|---|---|---|---|---|---|---|
| J 6—38 | *C. c.* | +— | 2,0 | 12 | 5 | 3 | | 4,22 | Hairs longer |
| c. pl. 1 | *C. p.* | 0 | 11,0 | 12 | 4 | 4 | | 4,18 | » » |
| 2 | *C. p.* | 0 | 9,5 | 8 | 7 | 5 | | 4,31 | » » |
| 3 | *C. p.* | 0 | 7,5 | 10 | 7 | 3 | | 4,21 | » » |
| 4 | *C. p.* | 0 | 9,0 | 9 | 9 | 2 | | 4,26 | » » |
| 5 | *C. p.* | 0 | 10,5 | 20 | | | | 4,02 | » » |
| 6 | *C. p.* | 0 | 11,0 | 16 | 4 | | | 4,11 | » » |
| 7 | *C. p.* | 0 | 8,5 | 4 | 12 | 4 | | 4,28 | » » |
| 8 | *C. p.* | 0 | 10,0 | 18 | 2 | | | 4,16 | » » |
| 9 | *C. p.* | 0 | 9,5 | 6 | 14 | | | 4,33 | » » |
| 10 | *C. p.* | 0 | 11,5 | 9 | 7 | 4 | | 4,36 | » » |
| 11 | *C. p.* | 0 | 13,0 | 16 | 4 | | | 4,08 | » » |
| 12 | *C. p.* | 0 | 8,0 | 12 | 6 | 2 | | 4,19 | » » |

Length of ligula in mm, of glumes in tenths of a mm. The hairiness of J 6—38 varies in different years. — *C. c.* = *Calamagrostis canescens*; *C. p.* = *Calamagrostis purpurea.*

Cytological studies in these doubled plants showed that they behaved as *C. purpurea* from Nature. No pollen was formed, and big plasmodia filled the loculi. On the female side the mitotic division occurred, the further evolution of the E.M.C. following the *Antennaria* scheme. The plants were not bagged, as they grew in a separate field far from other *Calamagrostis* species developing pollen. They gave seeds, from which seedlings were raised. As in the hybrid plants mentioned above, there were only 2—3 seedlings developed in each number. The chromosome numbers have not yet been counted. Still, the plants must be regarded as apomicts on account of their loss of pollen.

In my other experiments with *Calamagrostis* there is no analogy to this case, which may be due to the fact that these J 6—38 plants are the only doubled ones surviving in the cultures, except some doubled *Calamagrostis tenella* plants. Regarding the type of reproduction of the autopolyploids, this stands in opposition to the experiment of CHRISTOFF (1940) in *Hieracium leiophanum*, and I intend to repeat the experiments with more *C. canescens* biotypes to ascertain whether these also behave in the same way after doubling. In my cultures there is a *Calamagrostis villosa* biotype from Innsbruck with $2n = 56$. [*Calamagrostis villosa*

(CHAIX.) GMEL. is a Middle-European-Asiatic species which ordinarily has 2n = 28.] It stands very close to *C. canescens*. This plant breeds perfectly true, giving seeds even after bagging. It behaves like the apomicts in the genus, but, as far as I can see, it is an amphimictic species. The meiosis is perfectly regular with 28 bivalents on the male as well as on the female side, while *C. epigeios* with this chromosome number has plenty of tetravalents. Thus, the behaviour of various species within the genus with the chromosome number 56 is different, and for that reason no general conclusions regarding the relation between apomixis and autopolyploidy in the genus can be drawn until more information has been gathered.

## III. HYBRIDS BETWEEN APOMICTIC CALAMAGROSTIS PURPUREA AND AMPHIMICTIC SPECIES.

*Calamagrostis purpurea* commonly behaves as an obligate apomict by not developing pollen. In the E.M.C's a meiosis is completed only in rare cases (17,6 % of 119 investigated clones; NYGREN, 1946, p. 171) and later on the triads probably degenerate as in *Poa* (KIELLANDER, 1941; HÅKANSSON, 1943). In 1941, however, I got a *C. purpurea* plant (Gäll. 1—41) showing semiheterotypic division (ROSENBERG, 1926—27) on the male as well as on the female side besides mitotic divisions (NYGREN, 1946, pp. 156 and 171). The type of division could be controlled experimentally (p. 183). In 1944 crossings were made between this *C. purpurea* — as mother plant — and amphimictic species. In 14 combinations hybrids developed (l. c., p. 232). The hybrids flowered in the summer of 1947. I could immediately establish the fact that only the hybrids with the chromosome number 70, somatically, were developing panicles this summer. This was very striking in a cross with *Calamagrostis neglecta*. There were seven surviving plants, these having the chromosome numbers 70 (3 plants), 75, 78, 80 and 84. Only the three first-mentioned plants flowered. Thus a somewhat higher chromosome number delayed flowering. Besides the hybrids with 2n = 70, there was in the experimental field an unsuccessful »cross» comprizing 43 plants, where the egg-cell had developed autonomously, thus giving rise to agamospermous clone plants with 2n = 56. These plants were used as a control. It was very easy to verify that the true hybrids were darker all through than the clone plants. This applied to the green colour of the leaves as well as to the purple of the glumes. Values were worked out in the same manner as was described for synthetic

*C. purpurea* (p. 115). The differences were slight between the hybrids and the original clone. A most interesting find was that a descendant in a cross with *C. canescens* (221—3) had a glume length of only 4,00 mm, while the mother had 5,42 on an average. Plants with *C. neglecta* as father generally had shorter glumes than the mother clone. That this was not always the case can be seen from Table 3, Nos. 207—6 and 214—2. In some plants the ligulae are especially short, in No. 221—3 the values lie very close to the ideal one for *C. canescens* in this respect. It is important to note that hairs have appeared on the laminae in

TABLE 3. *Calamagrostis purpurea*: *crossings between Gäll. 1—41 and amphimicts.*

| Plant No. | Paternal species | Leaf hairs | Length of ligula | Length of glumes 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 | 63 | M (mm) | Relation lemma/callus hairs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 202—1 | *C. c.* | + | 9,0 | | | | 3 | 7 | 5 | 2 | 3 | | 5,43 | Hairs | longer |
| 2 | *C. c.* | + | 8,2 | | | | 4 | 6 | 2 | 5 | 3 | | 5,44 | » | » |
| 203 | *C. c.* | + | 6,7 | | | | | | 16 | 4 | | | 5,58 | » | » |
| 204—1 | *C. c.* | + | 8,2 | | | | | 8 | 4 | 6 | 2 | | 5,54 | » | » |
| 2 | *C. c.* | + | 9,5 | | | | 7 | 9 | 3 | 1 | | | 5,22 | » | » |
| 3 | *C. c.* | + | 9,5 | | | | 12 | 4 | 2 | 2 | | | 5,20 | » | » |
| 205 | *C. c.* | + | 11,5 | | | | 8 | 6 | | 4 | 2 | | 5,40 | » | » |
| 206—2 | *C. a.* | + | 9,5 | | | | 2 | 3 | 7 | 8 | | | 5,44 | » | » |
| 207—1 | *C. n.* | + | 7,5 | 8 | | 2 | 3 | 3 | 3 | 1 | | | 4,86 | 0 | |
| 5 | *C. n.* | + | 7,0 | | | | 16 | 3 | 1 | | | | 5,04 | Hairs | longer |
| 6 | *C. n.* | + | 10,0 | | | | | 14 | 4 | 2 | | | 5,36 | » | » |
| 208—1 | *C. c.* | + | 9,0 | | | | 12 | 4 | 2 | 2 | | | 5,22 | » | » |
| 214—1 | *C. n.* | + | 9,5 | | | | 18 | 2 | | | | | 5,02 | » | » |
| 2 | *C. n.* | + | 7,0 | | | | 7 | | 8 | | 5 | | 5,40 | » | » |
| 3 | *C. n.* | 0 | 11,1 | | 4 | 15 | 1 | | | | | | 4,58 | » | » |
| 215—1 | *C. v.* | + | 6,0 | | | 3 | 16 | 1 | | | | | 4,95 | » | » |
| 2 | *C. v.* | + | 6,0 | 1 | 1 | 3 | 15 | | | | | | 4,76 | » | » |
| 216—1 | *C. c.* | + | 7,0 | | | | | 13 | 4 | 2 | 1 | | 5,52 | » | » |
| 221—2 | *C. c.* | + | 7,5 | | | | 9 | 2 | 3 | 6 | | | 5,42 | » | » |
| 3 | *C. c.* | + | 4,5 | 20 | | | | | | | | | 4,00 | » | » |
| 222 | *C. a.* | + | 9,0 | | | | 3 | 11 | 5 | 1 | | | 5,26 | » | » |
| 223 | *C. c.* | + | 6,8 | | | | | 1 | 7 | 8 | 4 | | 5,76 | » | » |
| 225—1 | *C. a.* | + | 8,5 | | | | 1 | 3 | 14 | 2 | | | 5,50 | » | » |
| 2 | *C. a.* | + | 5,1 | | | | 2 | 5 | 7 | 3 | 5 | | 5,58 | » | » |
| 226 | *C. a.* | + | 8,0 | | | | 3 | 13 | 4 | | | | 5,20 | » | » |
| 220 1—43 | clone | 0 | 9,5 | | | 32 | 46 | 98 | 512 | 53 | 19 | | 5,40 | » | » |
| Gäll. 1—41 | | +— | 7,8 | | | | 5 | 6 | 7 | | 2 | | 5,42 | » | » |

Length of ligula in mm, of glumes in tenths of a mm. — *C. a.* = *Calamagrostis arundinacea*; *C. c.* = *Calamagrostis canescens*; *C. n.* = *Calamagrostis neglecta*; *C. v.* = = *Calamagrostis villosa.*

Nos. 206, 207, 214, 222, 225 and 226, while in these cases the paternal plants did not have any. The clone plants, No. 220, were smooth. The maternal plant, Gäll. 1—41, however, was hairy this summer as well as in 1944. All crosses with normally hairy *C. canescens* are hairy too. But in our botanical museums *C. purpurea* plants with hairy leaves are always classed as *Calamagrostis canescens* × *C. purpurea*, in spite of the fact in this actual case that crosses with the »smooth» species *C. arundinacea* and *neglecta* have also given hairy leaves. Thus it is evident that the mother plant itself (i. e. *C. purpurea*) determines the hairiness of the leaves. *C. neglecta*, a species with short callus hairs, has given one hybrid plant with callus hairs of the same length as the lemma (207—1). The other hybrids with *C. neglecta* show, however, that this is an exceptional case.

Cytological investigations in these hybrids indicate that they all behave like *C. purpurea* itself. Thus no pollen was formed, and the development of the E.M.C. corresponded to that of the *Antennaria* scheme. All plants were bagged and gave seeds abundantly. Consequently they are apomicts.

The confirmation of apomixis in these hybrids, however, does not permit the conclusion that apomixis is dominant in *Calamagrostis purpurea*. Then, leaving out of discussion the fact that only the progeny of one *C. purpurea* type has been investigated, all hybrids have received 56 chromosomes from their apomictic mother and only 14 from the amphimictic father (cf. GUSTAFSSON, 1947, p. 151).

The appearance of the hybrids agrees with the facts known from *Hypericum perforatum* and its hybrids with *H. maculatum* and *acutum* in this respect (NOACK, 1939). It indicates to the taxonomists the difficulty of determining hybrids of apomictic genera *exclusively* on the basis of what a plant looks like.

## IV. HAPLOID CALAMAGROSTIS PURPUREA ($2n = 28$).

(Figs. 2 and 13 respectively and 1 and 12 respectively.)

In a cross between the *C. purpurea* mentioned above, Gäll. 1—41, as mother and the *C. canescens* J 6—38 as father, I obtained in 1945 some descendants with the chromosome number $2n = 28$, besides agamospermous plants with $2n = 56$. The crossings were worked out in such a manner that a panicle from the »father», having been cut off and placed in a bottle of water beside the mother plant, was bagged together with a panicle from the latter. In spite of the fact that the

amphimictic species with 2n = 28 are allogamous, it happens now and then that the panicles cut off are able to produce some seeds. When the chromosome number of the 1945 descendants mentioned above was determined as 2n = 28, I, for that reason, suspected them to have sprung from seeds from the father, J 6—38. In the summer of 1947, however, one of the plants developed panicles, and it could easily be established that this particular individual had nothing to do with J 6—38. Certainly the plant has a short ligula, narrow leaves and anthers first yellow, later on purple, but the laminae are absolutely smooth, and the callus hairs are evidently shorter than the lemma, a character I have never seen in true *C. canescens*. The whole appearance of the plant deviates completely from what I have seen in the group Homeotricha of *Calamagrostis*. Still, if the plant is to be put in a species, *C. canescens* would be the only one that could come in question. It is, however, scarcely possible to expect haploid *C. purpurea* to be exactly of the same appearance as the *C. canescens* now existing, partly because *C. purpurea* has a polyphyletic origin, and partly because of the fact that *C. canescens*, which is included in the *C. purpurea* clone under investigation, could deviate in some way or other from the *C. canescens* biotypes in my cultures. In an amphimictic genus, viz. *Dactylis*, there is a similar case described by MÜNTZING (1943). — The values are given in Table 4.

TABLE 4. *Haploid Calamagrostis purpurea* (*2n = 28*).

| Field No. | Leaf hairs | Length of ligula | Length of glumes | | | | | | | | M (mm) | Relation lemma/callus hairs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 63 | | |
| 219 (h. p.) | 0 | 3,0 | | 3 | 12 | 5 | | | | | 4,26 | Palea longer |
| J 6—38 | +— | 2,0 | | 12 | 5 | 3 | | | | | 4,22 | Hairs » |
| Gäll. 1—41 | +— | 7,8 | | | | | 5 | 6 | 7 | 2 | 5,42 | » » |

Length of ligula in mm, of glumes in tenths of a mm. h. p. = haploid *C. purpurea*. J 6—38 = *Calamagrostis canescens*. Gäll. 1—41 = *Calamagrostis purpurea*.

The origin of this haploid must be due to haploid parthenogenesis. There is nothing in opposition to this in the development of the female side of Gäll. 1—41. The fact is that in certain panicles of Gäll. 1—41 meiosis occurs in the E.M.C's. In a cross with *C. canescens* I once obtained a hybrid plant with 2n = 48, as well as in another with *C. neglecta*, a descendant with 2n = 42, which indicates that a reduced egg-cell had taken part in the formation of the zygotes. In the haploid *C. purpurea* case the reduced egg-cell had obviously developed autonomously as in *Poa* (cf. KIELLANDER, 1942; HÅKANSSON, 1943).

Cytological investigations could not be made on account of the fixation being spoilt, but tests showed pollen to occur. It was, however, very poor and micro- and macro-grains lay mixed up in the loculi. Consequently the meiosis is probably as irregular as in most haploids known (TISCHLER, 1943, pp. 540—550).

## V. »CALAMAGROSTIS EPIGEIOS MUTATION» IN A CLONE OF CALAMAGROSTIS PURPUREA.

(Figs. 10 and 14 respectively; 11.)

In a *C. purpurea* clone (17) from Säter (1—38) there arose in 1946 a mutation involving the form of the panicle. In 1944, 39 plants, raised after bagging, were set out in the experimental field. They all had the chromosome number $2n = 56$. In the summer of 1946 I found that one of the plants (17—21) had panicles of the same agglomerated type as is so characteristic of *Calamagrostis epigeios* (Figs. 10 and 14). The other clone plants were the images of themselves, and all had the appearance of Säter 1—38. A panicle from one of them (17—3) is reproduced in Fig. 11. In further studies other distinguishing elements could be detected. Thus, the glumes of the mutation were shorter and broader near the base, and the hispidity of the glumes had not become so marked. Finally, what was most important of all, the callus hairs were shorter than the lemmas, just as was demonstrated in the haploid *C. purpurea*. The ligula and the hairiness did not vary. The values follow in Table 5.

TABLE 5. »*Calamagrostis epigeios mutation*» *in clone 17 of Calamagrostis purpurea.*

| Field No. | Leaf hairs | Length of ligula | Length of glumes 39 | 42 | 45 | 48 | 51 | M (mm) | Relation lemma/callus hairs |
|---|---|---|---|---|---|---|---|---|---|
| 17—21 (m) | 0 | 7,5 | 9 | 3 | 2 | 6 | | 4,37 | Lemma longer |
| 17— 3 | 0 | 7,2 | 8 | 5 | 7 | 3 | | 4,56 | Hairs » |
| 17—28 | 0 | 7,6 | 7 | 9 | 3 | 1 | | 4,44 | » » |

Length of ligula in mm, of glumes in tenths of a mm. m = mutation.

There are two important things characteristic of this plant. Firstly, the agglomeration of the panicles indicates the presence of genes for this character in the *C. purpurea* clone investigated. This may, of course, be due to a parallel variation in *C. purpurea* and *C. epigeios*. But, as the synthesizing experiments have shown *C. epigeios* to form a

part of some *C. purpurea* clones, the agglomeration in this case receives a very easy explanation. Secondly, the occurrence as well of short callus hairs in the mutation in question makes it very likely that more than one gene has been affected. The two acceptable parents of *C. purpurea*, viz. *C. canescens* and *C. epigeios*, have both long callus hairs, and the *C. purpurea* clone No. 17 ought thus to have a predisposition to long hairs from its father as well as from its mother.

## VI. DIFFERENT TYPES OF REPRODUCTION IN CALAMAGROSTIS PURPUREA CLONES.

In the summer of 1943 panicles from 40 different *C. purpurea* clones were bagged. The mother plants had been investigated earlier with regard to the type of division on the female side, and the *Antennaria* scheme had been ascertained for all of them. A control in 1944 gave the same results as also in 1945, when new facts concerning the relation of sexual and apomictic reproduction in *Calamagrostis purpurea* had been brought to light (NYGREN, 1946, p. 183). In 1944, 12 clone plants from each of the 40 numbers were set out side by side in rows, holding $3 \times 4$ plants. Besides these, there were planted 16, 21, 25, 35, 39 and 57 plants respectively from 6 numbers. In all cases the chromosome numbers of the descendants had been counted and confirmed to be the same as that of the mother clone. In 1945 only a few descendants developed panicles; some observations could, however, be made (cf. NYGREN, 1946, pp. 206—207). In 1946 I discovered the *C. epigeios* mutation just mentioned, as well as another very deviating plant from Robertsfors (100: 1—4). It was one and a half time as tall as the other sister plants in the clone, the leaves being broader and the glumes considerably longer than in the latter. The plant was definitely a gigas form in spite of having the same chromosome number as its sisters. Thus the gigantism must be due to mutation. I was surprised at the dark colour of the anthers and their swollen appearance in this plant, *C. purpurea* having as a rule yellow and thin anthers. An investigation showed that the gigas plant had plenty of well developed pollen grains in its loculi. A further study gave the divisions in the P.M.C's, following the semiheterotypical scheme of ROSENBERG (1926—27). When this became clear, I made an inventory of every clone plant included in the experiments. In 8 clones there were found 31 plants with pollen, distributed as seen in Table 6 below.

Five of these clones have been studied cytologically. All invest-

TABLE 6. *Distributions of plants developing pollen in 40 clones of Calamagrostis purpurea.*

| Field No. | Original locality | No. of plants | No. of plants with pollen |
|---|---|---|---|
| 42 | Gittafjäll | 12 | 0 |
| 46 | Luleå | 12 | 0 |
| 47 | Säter | 12 | 0 |
| 48 | Härnösand | 12 | 0 |
| 50 | Östersund | 12 | 0 |
| 51 | Härnösand | 12 | 0 |
| 52 | Klimpfjäll | 12 | 0 |
| 53 | Säter | 12 | 0 |
| 54 | » | 12 | 0 |
| 55 | Gittafjäll | 12 | 0 |
| 56 | Härnösand | 12 | 0 |
| 60 | Säter | 12 | 0 |
| 61 | » | 12 | 0 |
| 65 | Dorotea | 12 | 0 |
| 67 | Klimpfjäll | 12 | 0 |
| 69 | Säter | 12 | 0 |
| 70 | Jormlien | 12 | 0 |
| 71 | Härnösand | 12 | 2 |
| 76 | Stugun | 12 | 0 |
| 77 | Dalfors | 12 | 0 |
| 78 | Frösön | 12 | 0 |
| 79 | Vansbro | 12 | 0 |
| 83 | Härnösand | 12 | 0 |
| 84 | Säter | 12 | 0 |
| 85 | Jormlien | 12 | 0 |
| 86 | Klimpfjäll | 12 | 0 |
| 87 | Sylarna | 12 | 2 |
| 88 | Östersund | 12 | 2 |
| 89 | Dorotea | 12 | 9 |
| 90 | Säter | 12 | 0 |
| 91 | » | 12 | 0 |
| 92 | Gittafjäll | 12 | 5 |
| 93 | Säter | 12 | 0 |
| 94 | » | 12 | 0 |
| 95 | Jormlien | 12 | 5 |
| 96 | Härnösand | 12 | 0 |

| Field No. | Original locality | No. of plants | No. of plants with pollen |
|---|---|---|---|
| 97 | Klimpfjäll | 12 | 0 |
| 98 | Robertsfors | 12 | 0 |
| 99 | » | 12 | 3 |
| 100 | » | 12 | 3 |
| 1 | Säter | 25 | 0 |
| 17 | » | 39 | 0 |
| 20 | » | 57 | 0 |
| 24 | » | 16 | 0 |
| 27 | » | 35 | 0 |
| 36 | » | 21 | 0 |
| | | 673 | 31 |

igated plants showed meiotic as well as mitotic divisions in the E.M.C's. In plant 100: 1—1 there were 28 bivalents observed in metaphase I in the P.M.C's. As far as I can see, these plants behave like Gäll. 1—41, studied earlier; i. e. they propagate in an agamospermous manner and form restitution nuclei, which leaves the possibility open for crossing over followed by segregation in the offspring; they form hybrids with amphimictic species, and, finally, they give rise to haploids under certain conditions.

In order to investigate possible morphological properties »linked» to the presence of pollen, the measurements mentioned earlier were also worked out in these cases. As the plants resembled each other very much, the measurements did not include all »pollen plants», but, in-

TABLE 7. *Calamagrostis purpurea: comparison between »pollen-» and »non-pollen-plants» regarding morphology.*

| Field No. | Leaf hairs | Length of ligula | Length of glumes 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | M (mm) | Relation lemma/callus hairs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 87 1—1 x | 0 | 5,0 | | | | 9 | 5 | 6 | | | | 4,29 | Hairs longer |
| 1—4 | 0 | 8,0 | | | | | 4 | 7 | 6 | 3 | | 4,73 | » » |
| 2—1 x | 0 | 5,4 | | | | 8 | 6 | 2 | 4 | | | 4,36 | 0 |
| 2—4 | 0 | 5,3 | | | | 11 | 4 | 3 | 2 | | | 4,31 | 0 |
| 3—1 | 0 | 4,9 | | | | 3 | 5 | 8 | 4 | | | 4,46 | Hairs longer |
| 3—2 | 0 | 4,6 | 2 | 2 | 10 | 3 | 2 | 1 | | | | 4,17 | » » |
| 3—3 | 0 | 5,2 | | | | 4 | 6 | 8 | 2 | | | 4,49 | » » |
| 3—4 | 0 | 7,7 | | | | | | | 8 | 10 | 2 | 5,05 | » » |
| 88 1—1 | 0 | 6,2 | | | | 9 | 6 | 4 | 1 | | | 4,29 | » » |
| 1—2 x | 0 | 8,6 | | | | 2 | 3 | 5 | 9 | 1 | | 4,66 | » » |
| 2—1 x | 0 | 4,2 | | | | 3 | 5 | 12 | | | | 4,60 | » » |

| Field No. | Leaf hairs | Length of ligula | Length of glumes | | | | | | | | | M (mm) | Relation lemma/callus hairs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | | | |
| 2—2 | 0 | 7,1 | 1 | 1 | 2 | 3 | 1 | 12 | | | | 4,66 | » | » |
| 2—3 | 0 | 6,5 | | | 1 | 3 | 10 | 6 | | | | 4,66 | » | » |
| 2—4 | 0 | 9,8 | | | 3 | 1 | 4 | 12 | | | | 4,69 | » | » |
| 3—1 | 0 | 6,0 | | 2 | 8 | 2 | 2 | 6 | | | | 4,35 | » | » |
| 3—2 | 0 | 8,1 | | | 2 | 3 | 1 | 14 | | | | 4,81 | » | » |
| 3—3 | 0 | 7,4 | | | 7 | 4 | 4 | 5 | | | | 4,43 | » | » |
| 3—4 | 0 | 6,9 | | | 3 | 6 | 2 | 7 | 2 | | | 4,61 | » | » |
| 89 1—1 | 0 | 6,3 | | | 6 | 6 | 3 | 4 | 1 | | | 4,39 | » | » |
| 1—4 x | 0 | 12,3 | | | 20 | | | | | | | 4,20 | » | » |
| 3—3 | 0 | 8,1 | 5 | 7 | 8 | | | | | | | 3,84 | 0 | |
| 3—4 x | 0 | 7,5 | 3 | 3 | 10 | 4 | | | | | | 3,91 | 0 | |
| 92 1—1 | 0 | 15,0 | | | 4 | 7 | 5 | 3 | 1 | | | 4,36 | Hairs longer | |
| 1—2 x | 0 | 9,5 | | | | 1 | 3 | 12 | 3 | 1 | | 4,85 | » | » |
| 1—3 x | 0 | 9,0 | | 2 | 7 | 5 | 4 | 2 | | | | 4,29 | » | » |
| 2—2 x | 0 | 6,5 | | | 4 | 8 | 3 | 5 | | | | 4,43 | » | » |
| 2—4 x | 0 | 9,1 | | | 12 | 4 | 1 | 3 | | | | 4,38 | » | » |
| 3—1 | 0 | 12,0 | | | | 6 | 8 | 6 | | | | 4,54 | » | » |
| 3—2 | 0 | 8,0 | | | 7 | 8 | 5 | | | | | 4,22 | » | » |
| 3—3 | 0 | 9,7 | | 1 | 9 | 3 | 3 | 4 | | | | 4,36 | » | » |
| 95 1—1 x | 0 | 10,5 | | | | | 2 | 6 | 12 | | | 4,90 | » | » |
| 1—2 | 0 | 7,1 | | | | 4 | 15 | 1 | | | | 4,66 | » | » |
| 1—3 x | 0 | 9,0 | | | | 3 | 5 | 12 | | | | 4,52 | » | » |
| 2—2 | 0 | 10,0 | | 3 | 9 | 7 | 1 | | | | | 4,22 | » | » |
| 2—3 | 0 | 8,5 | | | | 7 | 13 | | | | | 4,44 | » | » |
| 3—2 x | 0 | 6,5 | 2 | 1 | 10 | 3 | 3 | 1 | | | | 4,24 | » | » |
| 99 1—1 | 0 | 6,8 | | 4 | 4 | 2 | 7 | 3 | | | | 4,35 | » | » |
| 1—2 | 0 | 6,6 | | 2 | 3 | 14 | 1 | | | | | 3,94 | » | » |
| 1—4 | 0 | 6,0 | | | | | | 3 | 6 | 7 | 4 | 5,42 | » | » |
| 2—1 | 0 | 6,2 | | 2 | 13 | 5 | | | | | | 4,11 | » | » |
| 2—2 | 0 | 6,4 | 1 | 1 | 14 | 2 | 2 | | | | | 4,08 | » | » |
| 2—4 x | 0 | 6,1 | | | 12 | 7 | 1 | | | | | 4,20 | » | » |
| 3—2 x | 0 | 8,0 | | | | | 3 | 17 | | | | 4,86 | » | » |
| 3—4 | 0 | 7,2 | | | 3 | 5 | 4 | 7 | 1 | | | 4,84 | » | » |
| 100 1—2 x | 0 | 7,0 | | | 16 | 4 | | | | | | 4,18 | » | » |
| 1—3 | 0 | 8,7 | | 3 | 15 | 2 | | | | | | 4,02 | » | » |
| 1—4 x | 0 | 11,5 | | | | 1 | 5 | 14 | | | | 4,84 | » | » |
| 2—1 | 0 | 9,3 | 2 | 1 | 15 | 2 | | | | | | 4,00 | » | » |
| 2—2 | 0 | 9,0 | | 2 | 10 | 6 | 2 | | | | | 4,23 | » | » |
| 2—3 | 0 | 9,3 | 2 | 1 | 17 | | | | | | | 3,96 | » | » |
| 2—4 | 0 | 7,2 | | 4 | 14 | 2 | | | | | | 4,02 | » | » |
| 3—2 x | 0 | 8,3 | | 1 | 19 | | | | | | | 3,98 | » | » |
| 3—3 | 0 | 7,1 | | | 5 | 15 | | | | | | 4,28 | » | » |
| 3—4 | 0 | 10,5 | | 2 | 9 | 8 | 1 | | | | | 4,20 | » | » |

Length of ligula in mm, of glumes in tenths of a mm. — Plants marked with x = plants with pollen.

stead, as many as possible of their pollenless sisters. Any differences between »pollen-» and »non-pollen-plants» could not be found, except in two cases: plant 100: 1—4, which gave very high values for the length of the glumes compared with the other clone plants, and plant No. 99: 1—4. The values can be seen in Table 7.

Thus it could be stated that no correlation existed between the presence of pollen in the anthers and the morphological appearance. The two cases, 99: 1—4 and 100: 1—4, which show such a correlation, very likely form exceptions.

## VII. DISCUSSION.

There are two types of species hybrids in *Calamagrostis* regarding the behaviour of meiosis. The first is the more common and embraces most hybrids between species with $2n = 28$. Here meiosis is quite regular on the male as well as on the female side (NYGREN, 1946, p. 238). The second type comprises a few hybrids on the 28-chromosomal level, e. g. some *C. canescens* × *C. epigeios* and all hitherto investigated interspecific hybrids on the 42- and 56-chromosomal one. A characteristic feature of these types is the almost unexceptional breakdown of meiosis on the male side, the P.M.C's forming plasmodia in the loculi. This has been confirmed for *C. arundinacea* × *C. epigeios* ($2n = 42$), *C. canescens* × *C. epigeios* ($2n = 28$, 42 and 56), as well as for *C. epigeios* × *C. pseudophragmites* ($2n = 42$). On the female side the situation is not so simple. A more or less regular meiosis has been found in the *C. arundinacea* hybrid mentioned above, and in *C. canescens* × *C. epigeios* ($2n = 28$ and 42). Besides this, there occur mitotical divisions (STEBBINS, 1932) in *C. arundinacea* × *epigeios* and in *C. canescens* × *epigeios* ($2n = 56$). (The *C. epigeios* × *pseudophragmites* hybrid has not yet been investigated in this respect.) The mitotical divisions are always much delayed and the egg-cells have only now and then the change of developing autonomously. Thus, the low percentage of seedlings in $F_2$ receives an explanation. This means, however, very little, as all *Calamagrostis* plants hitherto investigated are perennials. Moreover, I think that the chances are greater in the next generation for the formation of seeds by an agamospermous process. Apomictic $F_1$ plants occur in interspecific crosses of *Calamagrostis*. But how is this to be explained? In 1945 I could establish that the relation meiosis—mitosis in *C. purpurea* (Gäll. 1—41) was dependent on time (NYGREN, 1946, p. 182). In the panicles first developed meiosis was the rule, in the later panicles the divisions

were mitotical. In the hybrid *C. arundinacea* × *C. epigeios* (2n = 42) divisions occurred about two weeks later than in both parents, which was also found to be the case in *C. canescens* × *C. epigeios* (2n = 28) though here the flowering was only some days later than in *C. epigeios*. (*C. canescens* flowers two weeks earlier.) There must be some fundamental reason for this delay, as it does not apply to the rest of the hybrids on the 28-chromosomal level (including most combinations possible among European amphimictic species), nor in *C. epigeios* (2n = 42 = intraspecific hybrid, 28 × 56). I think that one of the reasons is the high chromosome number *in et per se*, but I do not believe that all interspecific hybrids in the genus with the chromosome numbers 42 and 56 *must* behave in such a manner. Many of them, however, do so. The lability of the division types has been proved in Gäll. 1—41, as well as in the experiments with the *C. purpurea* clones mentioned above. There is a disposition for sexual reproduction in many *C. purpurea* clones, but this does not always appear. Maybe, some hormones are able to change the late mitotic division in *Calamagrostis* into an early meiotic one. There *must* be something lacking when meiosis fails. The *C. purpurea* clone Gäll. 1—41 has given meiotical divisions every year between 1943 and 1946, but in 1947 I was not able to discover a single division, not even in the panicles first developed.

Not only the appearance of meiosis in *C. purpurea* seems to depend on mere chance, but also the presence of hairs on the laminae. The situation is the following: one year hairs can be found on all laminae of the plant, another year only on the uppermost leaves, and, finally, during a third one there are no hairs at all. The same is true of some of my cultivated *C. canescens* biotypes. There also exists a quantitative variation in hairiness, this applying to the number of hairs as well as for their length. Thus, there are, I think, some modifiers which act upon the behaviour of the hairs.

The taxonomical conclusions to be drawn from the crossing experiments between *C. purpurea* and amphimictic species open up no good prospects. When I began to investigate this species I had the fortune to find some very deviating plants, which could be interpreted as hybrids. With only one exception, the chromosome numbers of these plants were 2n = 70. The exception was a plant with 2n = 91, characterized by glumes of 7 mm length. In all clones in Nature I had no or small difficulties in distinguishing *C. purpurea* and its hybrids. The particular hybrid at hand was often difficult or sometimes impossible to determine. Actual experiments now show that in this case it is more or less

impossible to distinguish various hybrids between different amphimictic species and *C. purpurea* itself. The consequences of this situation are that we cannot maintain them as taxonomical units. There is nothing to do but simply to determine all such forms as *Calamagrostis purpurea* coll. In this giant, polyphyletic species the situation is the same as NANNFELDT found for *Poa arctica* (1940). Among a multitude of forms there are only a small minority that can be recognized sufficiently well to be described as subspecies and varieties. We must content ourselves with calling all other forms just *C. purpurea.*

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I am indebted to Professor A. MÜNTZING for his kindness in giving me working space at the Institute of Genetics at Svalöf and to Assistant C. G. ALM for taking the photographs presented in this paper.

## SUMMARY.

(1) In crosses between the amphimictic *Calamagrostis* species *canescens* ($2n = 28$) and *epigeios* ($2n = 42$), with the latter as mother, seven hybrid plants with the chromosome number $2n = 56$ have arisen. The hybrid plants look like the apomictic species *Calamagrostis purpurea* and are able to produce seeds after bagging in spite of the fact that their anthers do not produce any pollen. Thus they are synthetic apomicts.

(2) A situation resembling that described under (1) arose after colchicine treatment of seedlings of *C. canescens.* 12 doubled plants were developed; they all behave as *C. purpurea.* This case, however, needs further confirmation.

(3) Hybrids between *C. purpurea* and amphimictic species on the 28-chromosomal level all have the chromosome number $2n = 70$. They behave as pure *C. purpurea* in showing the *Antennaria* scheme on the female side.

(4) Hybrids with different amphimictic fathers could not be distinguished from each other.

(5) A haploid *C. purpurea* plant has been produced. It looks like *C. canescens* but deviates in some respects.

(6) A mutation in a *C. purpurea* clone shows the same agglomerated panicles as *C. epigeios.* This is not surprising, as *C. epigeios* forms a part of some of the *C. purpurea* clones.

(7) After bagging seedlings were raised from 40 *C. purpurea* clones having only mitotical divisions in their E.M.C's and never producing pollen. In the 673 descendants there arose 31 plants with pollen from 8 different clones. Plants from 5 of these clones were investigated and showed meiotic divisions on the female side as well.

(8) The divisions are retarded in *Calamagrostis* hybrids on the 42- or the 56-chromosomal level. This is expected to be one of the reasons why mitotic divisions occur in the E.M.C's of hybrids between amphimictic species.

(9) The appearance of hairs on the laminae varies very much in *C. canescens* and *C. purpurea* on the same plant in different years. This is considered to be due to modifiers.

(10) The taxonomical situation in *Calamagrostis purpurea* is stated to be the same as in *Poa arctica*. There are a few distinguishable types and a great many which cannot be kept apart.

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# THE SOMATIC CHROMOSOMES OF SOME TROPICAL PLANTS

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## INTRODUCTION.

IT is an interesting fact that the immense development of plant cytology during the last half century has been mainly made on material from temperate or arctic zones, while tropical plants have been rather stepmotherly treated. While only few blank spots occur in the lists of chromosome numbers where the former floras are concerned, many common tropical plants, let alone entire families, are still completely unknown cytologically. It is quite possible that this gap in our cytological knowledge may be of some general consequence and that many cytological phenomena will receive a different outlook when more is known of the cytology of tropical plants. Owing to the specific conditions to which tropical plants are adapted, and which bring about a cellular physiology deviating from conditions in plants from colder regions, it is conceivable that certain modifications of the cytological technique must be worked out in order to get the best possible results at the study of the chromosomes.

During his stay in the Scandinavian countries the present writer received some seeds from Java. At the revision of the chromosome numbers of this material certain interesting features were met with. Some other tropical plants, grown in the greenhouses of some botanical gardens the author visited in these countries, were therefore fixed and their root chromosomes studied. The present paper is a report of the somatic chromosome conditions of about 60 tropical plants, only some of which have been earlier investigated. In the following special section the arrangement of families and genera is in accordance with DARLINGTON and JANAKI AMMAL'S Chromosome atlas (1945). The following data are given for each species examined: chromosome number, chromosome size (measurements of the smallest and the largest chromosome of the idiogram), location of centromeric constrictions, when possible, and occurrence of satellites. A picture of a somatic metaphase plate in polar view is given from each species.

Seeds of seven of the species treated below and fixed root tips of

one species have been supplied through kindness of Dr. H. J. TOXOPEUS from the Hortus Bogoriensis, Java. The seeds were germinated on moist filter paper in Petri dishes and the primary root tips were fixed. The rest of the material consists of root tips collected from plants grown in pots in the greenhouses of Copenhagen, Lund, Gothenburg, Oslo and Bergen.

LEWITSKY, KARPECHENKO and some fixatives containing osmic acid were tried. Of these LEWITSKY and KARPECHENKO generally gave the best results. 2 BD followed by squashing and staining in FEULGEN (HILLARY, 1940) yielded very good results in the *Gloriosa* species and *Crinum*. This method, however, proved to be unsuitable for plants with many inclusions in the cytoplasm, for example, *Mimosa invisa*, *Leucaena glauca*, *Sesbania grandiflora*, *Amorphophallus titanum*.

Cross sections were cut at 14 $\mu$. In some cases inclusions in the cytoplasm made staining difficult also with other staining methods. Only in the case of *Pandanus* species and *Lantana camara* could bleaching of the sections in a solution of 1 vol. of 30 % peroxide and 10 vol. of 85 % alcohol improve the staining quality. The staining was almost exclusively made according to CLAUSEN's gentian violet method (OEHLKERS, 1940).

## SPECIAL SECTION.

1. *Fam. Piperaceae.* — *Piper longum* L. Not studied earlier. *Material*: Oslo, pot plant. $2n = 24$. *Chromosome size*: 0,8—2,0 $\mu$. Two chromosomes are longer than the rest. These two have clearly asymmetric arms. All analysable ones of the smaller chromosomes have median to submedian constriction. Two of them have a very pronounced constriction; it cannot be decided whether this constriction is a primary or a secondary one, delimiting a large satellite (Fig. 1 *a*).

2. *Fam. Sarraceniaceae.* — *Sarracenia Mitchelliania* and *S. purpurea* × *Tolliana*. Both are horticultural hybrids. No species of this family has been previously investigated. *Material*: Oslo, pot plant. $2n = 26$. *Chromosome size*: 2,0—3,5 $\mu$. Two chromosomes are somewhat longer; these are submedially attached. The rest of the chromosomes are also medially-submedially attached. One of the smaller chromosomes of *S. purpurea* × *Tolliana* has been found to have a fairly large satellite (Fig. 1 *b*, *c*).

3. *Fam. Portulacaceae.* — *Talinum paniculatum* GAERT. Earlier studied by SUGIURA (1936 a) under the synonymic name *T. patens*. This

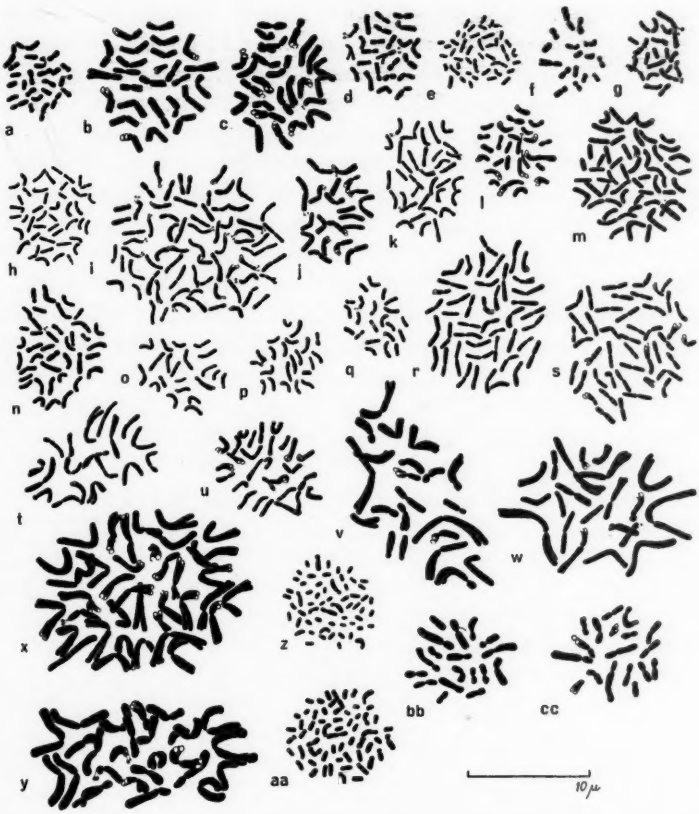


Fig. 1. *a—cc*: Polar views of mitotic metaphases. — *a*: *Piper longum* L. $2n = 24$. — *b*: *Sarracenia Mitchelliania*, hort. $2n = 26$. — *c*: *Sarracenia purpurea* × *Tolliana* $2n = 26$. — *d*: *Talinum paniculatum* GAERT. $2n = 24$. — *e*: *Lagerstroemia flos reginae* RETZ. $2n = 44$. — *f*: *Punica granatum* L. $2n = 16$. — *g*: *Flacourtia sepiaria* ROXB. $2n = 22$. — *h*: *Eugenia Cumini* MERRIL. $2n = 44$. — *i*: *Hibiscus radiatus* WILLD. $2n = 72$. — *j*: *Euphorbia tirucalli* L. $2n = 20$. — *k*: *Manihot carthaginensis* MUELL. $2n = 36$. — *l*: *Chrysophyllum cainito* L. $2n = 26$. — *m*: *Chrysophyllum oliviforme* LAM. $2n = 52$. — *n*: *Jasminum Sambac* SOLAND. $2n = 39$. — *o*: *Nerium oleander* L. $2n = 22$. — *p*: *Nerium odorum* SOLAND. $2n = 22$. — *q*: *Carissa edulis* VAHL. $2n = 22$. — *r*: *Lantana camara* L. $2n = 44$. — *s*: *Lantana polyacantha* SCHAU. $2n = 44$. — *t*: *Thunbergia alata* BOJ. $2n = 18$. — *u*: *Cryptocorine ciliata* BLUME. $2n = 28$. — *v*: *Gloriosa Rotschildiana* O'BRIEN. $2n = 22$. — *w*: *Gloriosa superba* L. $2n = 22$. — *x*: *Amorphophallus Rivieri* DUR. $2n = 39$. — *y*: *Amorphophallus titanum* BECC. $2n = 26$. — *z*: *Pandanus pacificus* VEITH. $2n = 60$. — *aa*: *Pandanus pygmaeus* THOU. $2n = 60$. — *bb*: *Curculigo recurvata* DRYAND. $2n = 18$. — *cc*: *Curculigo sumatrana* MIQ. $2n = 18$. — × 1850.

author found n = 12 in the PMC. *Material*: Copenhagen, pot plant. 2n = 24. *Chromosome size*: 1,5—2,0 $\mu$. Most of the chromosomes are more or less medially attached, a few, however, distinctly asymmetrically. One chromosome pair has satellites (Fig. 1 *d*).

4. *Fam. Lythraceae.* — *Lagerstroemia flos reginae* RETZ. Not hitherto examined. BOWDEN (1945 a) found 2n = 50 in *L. indica* L. var. *rubra* and *L. speciosa* PERS. *Material*: Lund, pot plant. 2n = 44. *Chromosome size*: 0,5—1,5 $\mu$. Stickiness was often observed in the material studied (Fig. 1 *e*).

5. *Fam. Punicaceae.* — *Punica granatum* L. This species has been chromosome-counted several times, and different numbers have been given. According to the current chromosome lists, n = 8, 2n = 18 and 19 (DARLINGTON and JANAKI AMMAL, 1945) have been determined. *Material*: Bergen, pot plant. 2n = 16. *Chromosome size*: 1—2 $\mu$. Two of the chromosomes are definitely larger than the others. These have submedian centromeres (Fig. 1 *f*).

6. *Fam. Flacourtiaceae.* — *Flacourtia sepiaria* ROXB. Of this family, commonly occurring in the tropics, only two species of *Hydnocarpus* seem to have been studied earlier (DARLINGTON and JANAKI AMMAL, 1945). *Material*: Copenhagen, pot plant. 2n = 22. *Chromosome size*: 1—2 $\mu$. Two of the largest chromosomes have subterminal centromeres, their shorter arms bearing satellites (Fig. 1 *g*).

7. *Fam. Myrtaceae.* — *Eugenia Cumini* MERRIL. Earlier studied by VAN DER PIJL (1934), who investigated the polyembryony of the genus and gave the chromosome number 2n = 42—44 and n = $\pm$ 20. Only three species of this economically important genus have been analysed cytologically. *Material*: Copenhagen, pot plant. 2n = 44. *Chromosome size*: 0,7—1,5 $\mu$. Chromosomes small, uniform and slender (Fig. 1 *h*).

8. *Fam. Malvaceae.* — This family has received pronounced attention by many writers because of the economical importance of several species. Three species of *Hibiscus* are considered here: *H. cannabinus* L., *H. radiatus* WILLD. and *H. Sabdariffa* L. Only *radiatus* is studied for the first time; the chromosomes of the other two have been described by SKOVSTED (1935, 1941).

*Hibiscus cannabinus* L. *Material*: seeds from S. Rhodesia, Nyasaland. 2n = 36. *Hibiscus radiatus* WILLD. and *H. Sabdariffa* L. *Material*: seeds from Java. 2n = 72. *Chromosome size*: 1,3—2,5 $\mu$. In *H. radiatus* (Fig. 1 *i*) at least 12 chromosomes quite regularly show

satellites, while in the other two species studied satellites were more seldom seen.

9. *Fam. Euphorbiaceae.* — *Euphorbia tirucalli* L. Previously unrecorded. *Material*: Lund, pot plant. $2n = 20$. *Chromosome size*: 1,5—3,0 $\mu$. Median to submedian centromeres. Two large chromosomes with median constrictions have satellites (Fig. 1 *j*). *Manihot carthaginensis* MUELL. Not studied earlier. *Material*: Oslo, pot plant. $2n = 36$. *Chromosome size*: 1,2—2,0 $\mu$. Chromosomes thus much smaller than in the preceding species. Two chromosomes with satellites (Fig. 1 *k*).

10. *Fam. Mimosaceae.* — Taxonomically this family must be considered as highly uniform and well defined. This is reflected in the chromosome numbers, which nearly all belong to the 13-series. There have been published two exceptions: $n = 24$ for *Mimosa pudica* (KAWAKAMI, 1930), and $2n = 24$ for *Albizzia lophantha* (SUGIURA, 1931). These numbers, however, are probably erroneous, as already pointed out by TISCHLER (1935/36) concerning the latter one. On account of the great botanical and practical interest of this family I decided to study several species of five of its genera. In all forms investigated the cytological features are rather concordant. This is seen from Fig. 2 *a—aa*, where root chromosomes of the species studied by me are pictured. A phenomenon which seems to be of general occurrence in the Mimosaceae hitherto investigated is the endomitotic doublings in the root meristem and the cortical tissue. It has been reported by GHIMPU (1929 a, b), NEWMAN (1934), SENN (1938) and EICHHORN (1938), and has been constantly encountered by me when the primary roots of the seedlings have been under examination. In secondary roots, on the other hand, endomitotic duplications have never been observed in my material. Between the primary root and later-formed roots there is evidently some anatomical or physiological difference which favours the origin of polysomatic mitoses in the former. Another feature commonly occurring in the Mimosaceae is persisting nucleoli, as has already been noted in the literature (EICHHORN, l. c.).

*Mimosa biuncifera* BENTH. Not studied earlier. *Material*: Copenhagen, pot plant. $2n = 52$. *Chromosome size*: 1—2 $\mu$. At least five chromosomes with satellites. (Fig. 2 *a*).

*Mimosa pudica* L. Investigated by KAWAKAMI (l. c., see above). *Material*: Copenhagen, Lund, Oslo, pot plants and seeds. $2n = 52$. *Chromosome size*: 1,0—1,5 $\mu$. No satellites seen (Fig. 2 *b*). The seedlings regularly contain polysomatic cells, the chromosomes of which were often sticky.

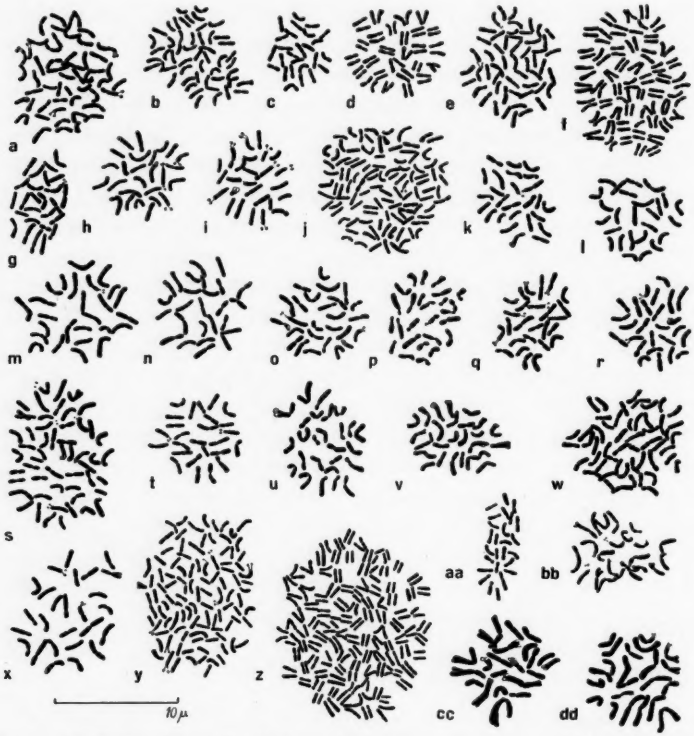


Fig. 2. *a—dd*: Polar views of mitotic metaphases. — *a*: *Mimosa biuncifera* BENTH. 2n = 52. — *b*: *Mimosa pudica* L. 2n = 52. — *c—f*: *Mimosa invisa* MART. *c*: monosomatic 2n = 26, *d*: disomatic 2 × 2n = 52 paired, *e*: disomatic 2 × 2n = 52 unpaired, *f*: *tetrasomatic* 2 × 2 × 2n = 104 paired. — *g*: *Albizzia lophantha* BENTH. 2n = 26. — *h*: *Albizzia Neumannia* HEYNE. 2n = 26. — *i*: *Albizzia procera* BENTH. 2n = 26. — *j*: *Albizzia polyphylla* FOURN. 2n = 104. — *k*: *Acacia alata* R. BR. 2n = 26. — *l*: *Acacia armata* HEYNE. 2n = 26. — *m*: *Acacia Baileyana* MUELL. 2n = 26. — *n*: *Acacia cyanophylla* LINDL. 2n = 26. — *o*: *Acacia falcata* WILLD. 2n = 26. — *p*: *Acacia glaucoptera* BENTH. 2n = 26. — *q*: *Acacia graveolens* A. CUNN. 2n = 26. — *r*: *Acacia longifolia* WILLD. 2n = 26. — *s*: *Acacia Koa* A. GRAY. 2n = 52. — *t*: *Acacia melanoxylon* ROXB. 2n = 26. — *u*: *Acacia penninervis* SIEBER. 2n = 26. — *v—w*: *Acacia retinodes* A. CUNN. *v*: monosomatic 2n = 26, *w*: disomatic 2 × 2n = = 52. — *x*: *Acacia verticillata* WILLD. 2n = 26. — *y—z*: *Leucaena glauca* BENTH. *y*: monosomatic 2n = 104, *z*: disomatic 2 × 2n = 208 paired. — *aa*: *Enterolobium timbouva* MART. 2n = 26. — *bb*: *Cajanus indicus* SPRENG. 2n = 22. — *cc*: *Derris pubipetale* MIQ. 2n = 22. — *dd*: *Derris uliginosa* BENTH. 2n = 22. — × 1850.

*Mimosa invisa* MART. Not recorded earlier. Material: Java, seeds. 2n = 26. Evidently both diploid and tetraploid species occur in *Mimosa*. *Chromosome size*: 1,0—1,5 $\mu$. Satellites have been seen in

some plates. Polysomatic cells are frequent, both tetraploid and octoploid cells with their chromosomes arranged in pairs and unpaired, representing various stages in the endomitotic doubling (Fig. 2 *c*—*f*).

*Albizzia lophantha* BENTH. Studied by SUGIURA (l. c., cf. above). *Material*: Copenhagen, Lund, pot plants. 2n = 26. *Chromosome size*: 1,3—2,0 $\mu$. Two satellites in some plates (Fig. 2 *g*).

*Albizzia Neumannia* HEYNE. Not studied earlier. *Material*: Lund, pot plants. 2n = 26. *Chromosome size*: 1,3—1,7 $\mu$. Some chromosomes with satellites (Fig. 2 *h*).

*Albizzia procera* BENTH. Previously uninvestigated. *Material*: Lund, pot plants. 2n = 26. *Chromosome size*: 1,3—1,7 $\mu$. Many chromosomes (at least 7) with satellites (Fig. 2 *i*).

*Albizzia polyphylla* FOURN. Not studied earlier. *Material*: Lund, pot plants. 2n = 104. *Chromosome size*: 0,7—1,8 $\mu$. Satellites occur. This form is evidently an octoploid species. The somatic number 104 was found all through several root tips examined and cannot be due to polysomaty (Fig. 2 *j*).

*Acacia*. 14 species have been investigated, among them 13 Australian and one from the Sandwich Islands. The Australian species are mentioned below, followed by a reference to the source from which the material was obtained:

| | | | |
|---|---|---|---|
| 1. | *Acacia alata* R. BR. | Lund | Fig. 2 *k* |
| 2. | » *armata* HEYNE | Copenhagen | » 2 *l* |
| 3. | » *Baileyana* MUELL. | Gothenburg | » 2 *m* |
| 4. | » *cyanophylla* LINDL. | Lund | » 2 *n* |
| 5. | » *falcata* WILLD. | Copenhagen | » 2 *q* |
| 6. | » *glaucoptera* BENTH. | Lund | » 2 *p* |
| 7. | » *graveolens* A. CUNN. | » | » 2 *o* |
| 8. | » *longifolia* WILLD. | Gothenburg | » 2 *r* |
| 9. | » *melanoxylon* ROXB. | » | » 2 *t* |
| 10. | » *penninervis* SIEBER | Copenhagen | » 2 *u* |
| 11. | » *retinodes* A. CUNN. | » | » 2 *v*, *w* |
| 12. | » *verticillata* WILLD. | » | » 2 *x* |
| 13. | » *xylocarpa* A. CUNN. | Lund | (not pictured) |

Among these Nos. 1, 3, 4 and 8 have been studied earlier by EICHHORN (No. 1), NEWMAN (No. 3) and GHIMPU (Nos. 4 and 8), all the others are here investigated for the first time. All the species have 2n = 26. Their chromosome size ranges from 1,5—2,5 $\mu$. As will be seen from the above-mentioned figures, the appearance of the chromo-

somes agrees fairly well in all of them. A number of species, *e. g.*, Nos. 5, 7, 8, 9, 13 of the above enumeration, have clear satellites. Some fixations were very favourable, as for instance Fig. 2 *x*, which picture allows of a location of the centromeric constrictions in most chromosomes.

Polysomaty was found in *A. retinodes*, this being the only species in which both primary roots and older roots were fixed. Polysomatic mitoses were observed only in the former roots. A striking case of spontaneous stickiness was found in *A. cyanophylla* with resulting non-congression, bridge formation and lagging anaphase chromosomes.

*Acacia Koa* A. GRAY. This species occurring in the Sandwich Islands was fixed on material from Gothenburg. Not previously studied. $2n = 52$, thus a tetraploid species. The chromosome size and appearance are in agreement with the Australian species investigated. Four chromosomes bearing satellites were seen (Fig. 2 *s*).

*Leucaena glauca* BENTH. Not studied earlier. *Material*: Java, seeds. $2n = 104$. *Chromosome size*: 0,7—1,6 $\mu$. Chromosomes somewhat smaller than the *Acacia* chromosomes, but in agreement with the 104-chromosome *Albizzia polyphylla*. At least 7 chromosomes with satellites (Fig. 2 *y*). Endomitosis occurs regularly, one plate with 208 chromosomes arranged in pairs being pictured in Fig. 2 *z*.

*Enterolobium timbouva* MART. Genus previously uninvestigated. *Material*: Lund, pot plant. $2n = 26$. *Chromosome size*: 0,7—1,7 $\mu$. The chromosomes are the smallest ones found by me among Mimosaceae. No satellites observed (Fig. 2 *aa*).

11. *Fam. Papilionaceae, a. Tribe Galegae. — Sesbania grandiflora* PERS. Studied earlier by KRISHNASWAMI and RANGASWAMI AYYANGAR (1935 a), who gave 7 as haploid number, and by JACOB (1941), who gave $n = 12$ and $2n = 24$. *Material*: Java, seed. $2n = 24$. *Sesbania sesban* MILL. Studied by JACOB (l. c.). *Material*: Java, seed. $2n = 12$. Pictures of the two *Sesbania* species are not given here, since my pictures are in perfect agreement with those given by JACOB. Like this author, I also found non-congression, chromosome lagging and bridges, caused by the often noticed stickiness. JACOB pointed out that the basic number of the genus is 6, and my results agree with this. The basic numbers 7 and 8 occurring in the Chromosome atlas (DARLINGTON and JANAKI AMMAL, 1945) are presumably erroneous.

*b. Tribe Phaseolae. — Cajanus indicus* SPRENG. ROY (1933) and KRISHNASWAMI and RANGASWAMI AYYANGAR (1935 b) give the chromosome number $n = 11$. *Material*: Copenhagen, pot plant. $2n = 22$.

*Chromosome size*: 1,6—2,0 $\mu$. Chromosomes slender, median to submedian attachment. Four chromosomes with very clear satellites (Fig. 2 *bb*).

*c. Tribe Dalbergeae. — Derris pubipetale* MIQ. Not earlier studied. *Material*: Java, fixed root tips. 2n = 22. *Chromosome size*: 2,0—4,2 $\mu$. Two chromosomes are larger than the rest. Most chromosomes have median-submedian attachment (Fig. 2 *cc*).

*Derris uliginosa* BENTH. Previously unrecorded. *Material*: Copenhagen, pot plant. 2n = 22. The chromosomes are of the same size and appearance as in the preceding species. Two of the smaller chromosomes have small satellites on their short arms (Fig. 2 *dd*).

12. *Fam. Sapotaceae. — Chrysophyllum cainito* L. and *Chr. oliviforme* LAM. Of the entire family only one species of each *Palaquium* and *Achras* seems to have been studied earlier. *Material*: Gothenburg, pot plant. 2n:*cainito* is a diploid with 26 chromosomes, *oliviforme* is a tetraploid with 52 chromosomes. *Chromosome size*: 1—3 $\mu$ in both. The general type of chromosomes is similar in the two species. In *oliviforme* stickiness was often observed (Fig. 1 *l*, *m*).

13. *Fam. Oleaceae. — Jasminum Sambac* SOLAND. This species has been examined by BOWDEN (1945 b), who found 2n = 26. *Material*: Copenhagen, Bergen, pot plant. 2n = 39. This type is evidently a triploid form of the species. *Chromosome size*: 1,0—1,7 $\mu$. Most of the chromosomes have median to submedian constriction, but some also have subterminal. One chromosome with a satellite has been seen in several plates (Fig. 1 *n*).

14. *Fam. Apocynaceae. — Nerium oleander* L. and *N. odorum* SOLAND. SUGIURA (1931) found 2n = 22 in *oleander*, SCHÜRHOFF and MÜLLER (1937) report n = 8 for this species. *Odorum* has been studied by SUGIURA (1936 b) and given n = 11. *Material*: Copenhagen, Lund (*oleander*), Lund (*odorum*), pot plants. 2n = 22 in both. *Chromosome size*: 1—2 $\mu$. Median-submedian constrictions (Fig. 1 *o*, *p*).

*Carissa edulis* VAHL. Not recorded earlier. *Material*: Lund, pot plant. 2n = 22. *Chromosome size*: 0,8—1,5 $\mu$. Median-submedian attachment. Satellites have been observed in some plates (Fig. 1 *q*).

15. *Fam. Verbenaceae. — Lantana camara* L. and *L. polyacantha* SCHAU. (possibly the same species). Not previously studied. *Material*: Lund, pot plants. 2n = 44. *Chromosome size*: 1,5—2,5 $\mu$. The chromosomes are similar in both species, most of them having median-submedian attachment (Fig. 1 *r*, *s*).

16. *Fam. Acanthaceae. — Thunbergia alata* BOJ. Examined be-

fore by SUGIURA (1931), who found n = 9. *Material*: Java, seeds collected from wild-growing plants. 2n = 18. *Chromosome size*: 2—4 $\mu$. This species is a favourable cytological material, as the chromosomes stain readily, are slender and of clear morphology. The following chromosome types are recorded: two large chromosomes with median attachment, several medium-sized with median-submedian attachment, four of the smaller chromosomes have subterminal attachment (Fig. 1 *t*).

17. *Fam. Aroideae.* — *Cryptocorine ciliata* BLUME. The family completely unknown cytologically. *Material*: Oslo, pot plant. 2n = 28. *Chromosome size*: 1,5—2,7 $\mu$ (Fig. 1 *u*).

18. *Fam. Liliaceae. Tribe Uvularieae.* — *Gloriosa Rotschildiana* O'BRIEN and *G. superba* L. TISCHLER (1931) gives the number 2n = 22 for *Gloriosa superba*, the same number is assigned by JANAKI AMMAL to *G. Rotschildiana* (DARLINGTON and JANAKI AMMAL, 1945). No chromosome pictures have been published earlier of these species. *Material*: Lund, Bergen (*Rotschildiana*), Bergen (*superba*), pot plants. 2n = 22. *Chromosome size*: Two large chromosomes with median attachment, four medium with median-submedian attachment, two medium with subterminal attachment, these latter have small satellites on their short arms, 14 chromosomes are smaller, some of them are distinctly asymmetric (Fig. 1 *v*, *w*).

19. *Fam. Araceae.* — *Amorphophallus Rivieri* DUR. and *A. titanum* BECC. These two species have not been studied earlier. For other species, however, the chromosome lists give the diploid number 26 and in one case 28 in a species for which 26 is also given. *Material*: Oslo, Lund (*Rivieri*), Lund (*titanum*). 2n:*titanum* is a diploid with 26 chromosomes, while *Rivieri* is a triploid with 39 chromosomes. *Chromosome size*: The diploid seems to have somewhat larger chromosomes than the triploid, the chromosomes ranging from 3 to 6,5 $\mu$ in the former and from 4 to 7,5 in the latter. Most of the chromosomes have median to submedian attachment, two chromosomes of each genome have clearly subterminal attachment, one of these has a satellite on its shorter arm. In the triploid no more than one satellited chromosome has been observed in the same plate (Fig. 1 *x*, *y*).

20. *Fam. Pandanaceae.* — *Pandanus pacificus* VEITH. and *P. pygmaeus* THOU. The family not investigated earlier. *Material*: Lund, pot plants. 2n = 60. *Chromosome size*: 0,2—1,5 $\mu$. In *pacificus* six chromosomes are distinctly larger than the others; they have subterminal constrictions (Fig. 1 *z*). Conditions are less clear in *pygmaeus* (Fig. 1 *aa*).

21. *Fam. Hypoxidaceae.* — *Curculigo recurvata* DRYAND and *C. sumatrana* MIQ. In DARLINGTON and JANAKI AMMAL (1945) the numbers 2n = 18 and c. 50 are given for two *Curculigo* species. In the paper by SATO (1938) from which these numbers originate I have been unable, however, to find other reference to *Curculigo* than the information about the basic number = 9 and 10 (l. c., p. 228). *Material*: Oslo (*recurvata*), Lund (*sumatrana*), pot plants. 2n = 18. *Chromosome size*: 1,5—3,5 $\mu$. Two large chromosomes with subterminal attachment, the rest have median to subterminal attachment (Fig. 1 *bb*, *cc*).

## CONCLUSIONS.

In the present paper root chromosomes of 59 species of 29 genera belonging to 21 families have been studied. Special attention has been directed to Mimosaceae, in which 23 species of 5 genera have been investigated. Of the 59 species examined, the chromosome numbers of 38 species have not been reported previously. Polysomaty was observed in the cortex of the primary roots of seedlings in *Mimosa*, *Acacia* and *Leucaena*.

This investigation emphasizes the striking lack in our knowledge of the chromosome conditions of tropical plants.

In conclusion I wish to express my gratitude to Professor C. A. JØRGENSEN and Mr. G. GUDJONSSON of the Genetical Institute of the Royal Veterinary and Agricultural College, Copenhagen, who helped and inspired me to start the present study, and to Dr. A. LEVAN, Svalöf, for suggestions and criticism during my work at the Swedish Seed Association. I am also greatly indebted to the authorities of the different botanical gardens for allowing me to use their precious tropical plants.

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# MEIOSIS AND POLYPLOID CHARACTERS IN THE TETRAPLOID APPLE VARIETY HIBERNAL

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## I. INTRODUCTION.

AMONGST the wild species of the genus *Malus* several tetraploid forms are known. NEBEL (1929) was the first to report the chromosome number 2n = 68 in two different species, viz. *M. coronaria* and *M. glaucescens*. Later the same number was also found in *M. platycarpa* and *M. glabrata* (LINCOLN and MC CANN, 1937). On the other hand, tetraploids are very rare among the cultivated apple varieties. The first established as such seems to have been the variety Kola created by HANSEN. Its chromosome number was counted by NEBEL (1929). It is an artificial hybrid between a wild North-American *Malus* species (*M. ioense*) and the cultivated variety Charlamowsky. The hybrid represents a crab-apple type of rather small economic value.

Recently, since the great significance of the tetraploid forms in producing triploid and possibly valuable apple varieties was stressed (NILSSON-EHLE, 1938), an extensive breeding work with this object in view has begun, especially in Sweden. Huge quantities of seeds collected from various triploid apple varieties were sown, derived partly after open pollination, partly after artificial crossings between the triploids and certain diploids. In this material a few plants were very soon found with 68 somatic chromosomes (JOHANSSON, 1937; BERGSTRÖM, 1938). Colchicine treatment of seeds has also led to similar results, i. e. tetraploid apple seedlings (NILSSON and LARSSON, 1944). The material has quickly increased when the tetraploids have reached the flowering stage. A great number of investigations have been published of such findings (NILSSON-EHLE, 1942, 1944; ASCHÁN, 1943, NILSSON and LARSSON, 1944; JOHANSSON, 1944; OLDÉN, 1945, 1946).

In these works, however, an already existing and early cultivated tetraploid apple variety seems to have been totally ignored. It should not be forgotten that so far back as 1939 LINCOLN in a short account

reported that the variety Hibernal has a tetraploid chromosome number of $2n = 68$.

HEDRICK (1938) gives us some information of the history and properties of this variety. It originated in Russia about 1870—80 and was later imported into the U. S. A. It is quite commonly cultivated in orchards in the most extreme regions of the U. S. A. and in Canada, being quite healthy, hardy and productive even under very cold climatical conditions.

Owing to the initiative of Professor O. MEURMAN the variety in question has been added to the collection of apple trees at the Horticultural Institute at Piikkiö, having been acquired from a Finnish nursery which in its turn had imported the original plant from Canada. MEURMAN has described the variety in his pomology (MEURMAN and COLLAN, 1943). The trees have proved to be quite hardy in Finland, similar to their behaviour in North-America. In fact, Hibernal was one of the few apple trees which survived the extremely hard winter of 1939—40. In addition to this the variety grows vigorously, with stout and short branches. The fruit is rather large with coarse flesh and thick skin. The leaves are thick and dark-green. In annual yield Hibernal has been rather good.

Our knowledge of meiosis in tetraploid *Malus* species is based in the main on the rather scanty observations made by NEBEL (1929). Meiosis of the newly created tetraploid seedling apples was thus far wholly uninvestigated. However, for possible breeding work it was necessary to verify with certainty the tetraploid nature of the variety Hibernal. An analysis of the main features of its meiosis has therefore been performed, the results of which will be given below. In addition some morphological properties elucidating the character of its polyploidy have also been studied.

## II. MATERIAL AND METHODS.

The material was collected from a tree growing in the experimental garden of the State Horticultural Institute at Piikkiö. This tree is now twelve years old and grafted on a seedling root-stock. A number of branches cut during the spring were kept in water jars in the laboratory, the buds being fixed at proper time. They were first dipped in Carnoy solution and subsequently fixed in craf solution according to RANDOLPH. The slides were stained by using crystal violet. Especially the chromosomes at first metaphase were difficult to stain well. The staining

process was therefore prolonged up to five hours in 0,5 per cent solution of the dye. After that the handling of the slides was continued according to the chromic modification for deeper staining, proposed by LA COUR (DARLINGTON and LA COUR, 1942, p. 123).

## III. RESULTS.

### 1. MEIOSIS.

The most characteristic feature of meiosis in the variety Hibernal is the abundant formation of multivalents, a finding that is, as known, quite common especially in autotetraploid plants. The multivalent groups can be already observed at prophase. Thus at pachytene numerous changes of partners in the conjugating chromosomes can be found. This stage is, however, not suitable for more critical analysis owing to the high number of chromosomes. At diakinesis (Fig. 1) several multivalent chromosome associations can be seen. For a more detailed examination of these associations, however, diakinesis is not sufficiently distinct. The contraction of chromosome is, compared to the normal one, rather incomplete. A similar situation has earlier been met with in certain other autotetraploid plants also (cf. MYERS, 1945; VAARAMA, 1947).

Fig. 1. Diakinesis. Two univalents and different multivalent chromosome associations. — × 2800.

At metaphase I the study of the chromosome associations could be made with sufficient accuracy, although the great number of units and their small size somewhat hampered the examination.

A total of 15 PMC's was analysed. In all cases the full chromosome number was found to be 68. Besides univalents these PMC's contain a varying number of different chromosome associations from bivalents up to nonavalents. Fig. 2 shows the whole chromosome complement of one PMC, the different chromosome configurations being drawn separately. The cell in question is characterized by the unfamiliar high rate of multivalent associations. In Table 1 the variance and the average values of different chromosome associations are given from the analysed group of 15 PMC's. Furthermore, the percentage of chromosomes in each group, calculated as pairs, will be found in the last row.

TABLE 1. *The frequency of the different chromosome associations at metaphase I.*

| | The valency of the configurations | | | | | | | | | Total of pairs |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | |
| Variance per one PMC ..... | 1—6 | 0—8 | 0—7 | 4—11 | 0—5 | 0—3 | 0—1 | 0—1 | 0—1 | — |
| Average per one PMC ..... | 2,73 | 4,07 | 3,01 | 6,73 | 2,20 | 1,13 | 0,27 | 0,07 | 0,07 | — |
| Percentage calculated as pairs ..... | 4,0 | 12,0 | 13,4 | 39,6 | 16,2 | 10,0 | 2,8 | 0,8 | 0,8 | 510 |

Fig. 2. The chromosome set of a PMC with especially high multivalent formation. Different chromosome associations drawn separately: $2_{I}$, $2_{III}$, $6_{IV}$, $3_{V}$, $2_{VI}$, $1_{IX}$. — Fig. 3. Multivalent types from different PMC's. *a*, the commonest trivalent type. *b*—*c*, different tetravalent types. *d*—*h*, pentavalents. *i* and *j*, hexavalents, *k*, septa-, *l*, octo- and *m*, nona-valent. — × 2800.

From Table 1 it can be observed that tetravalents are the most common. The formation of bivalents is proportionally weak in comparison with the conditions in some other autotetraploid plants reported earlier (e. g. UPCOTT, 1935; VAARAMA, 1947). Trivalents and pentavalents are more common. The number of hexavalents seems to be about the same as that of bivalents. The rarest are configurations with 7, 8 or 9 units. Nonavalents here form the highest association, as is also the case in the triploid apple varieties (DARLINGTON and MOFFETT, 1930).

In almost all cells univalents are present. Their number is, however, not always correlated to the number of uneven chromosome associations. This has also been observed in the colchicine-induced tetraploid *Ribes nigrum* (VAARAMA, 1947). In different cells from one to six univalents, similar to the condition reported by DARLINGTON and MOFFETT (l. c.), are found. Table 2 shows the frequency of univalents in 65 first anaphase cells.

TABLE 2. *The frequency of univalents at the first division anaphase.*

| Number of univalents | 0 | 1 | 2 | 3 | 4 | 5 | 6 | Total |
|---|---|---|---|---|---|---|---|---|
| Number of PMC's ..... | 4 | 10 | 18 | 13 | 10 | 6 | 4 | 65 |

The chiasmata at metaphase I are mostly completely terminalized. On account of the small size of the chromosome the formation of chiasmata is very abundant. A detailed study of the multivalent configurations makes this quite obvious. It may also be stated that associations involving the formation of more than one chiasma in one and the same chromosome arm are unexpectedly common. In order to elucidate this situation the frequencies of the different types of 47 trivalents and 101 tetravalents (types classified according to DARLINGTON, 1931) present in the 15 PMC's analysed are collected in Table 3.

Values found for the different types compared with the corresponding ones in some other autotetraploid plants furnished with chromosomes of about the same size (UPCOTT, 1935; VAARAMA, 1947) reveal, in fact, that the chiasma formation here is unusually strong. The average chiasma frequency, calculated as half chiasmata, is 1,89 per unit.

TABLE 3. *The percentage of the different tri- and tetra-valent types.*

| Association type | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of type according to DARLINGTON (1931)... | 7 | 8 | 9 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| Percentage of the type..... | 21,3 | 17,0 | 61,7 | 14,9 | 3,0 | 5,0 | 6,0 | 7,9 | 20,9 | 26,9 | 15,9 |

In addition to the association types present in the PMC drawn in Fig. 2 a number of different multivalent configurations encountered in the material studied are to be found in Fig. 3. The types of these multivalents correspond to those described earlier in the tribe Pomoidae (DARLINGTON and MOFFETT, 1930; MOFFETT, 1931). The comparatively high chiasma frequency leads to the common occurrence of ring bivalents as important components within the multivalent configurations.

At I—A the conjugated chromosomes are distributed first. The uni-

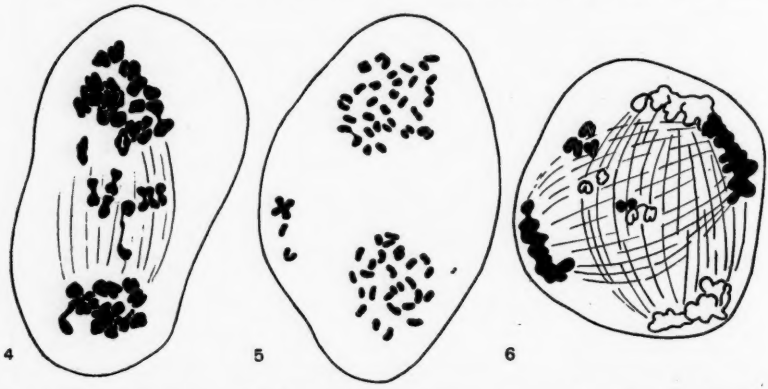


Fig. 4. Anaphase I. Six dividing univalents between the poles. One bivalent is lagging. The connection between some chromosomes has remained after segregation. In the upper group one ring bivalent. — Fig. 5. Anaphase II (the lower chromosome group has not been drawn). The plates with 35 and 27 chromosomes. To the left several eliminations from first division. — Fig. 6. Telophase II. In the upper spindle (black) one dividing chromosome. In the spindle below (white) two free lagging chromosomes and in addition two others connected with one another. The striped chromosomes are eliminations from first division. — × 2100.

valents in their turn are found lagging and divide at the time the other chromosomes have already reached the poles (Fig. 4). The same is true of the diploid apples and of other species of the tribe (DARLINGTON and MOFFETT, l. c.; MOFFETT, l. c.). The chromosomes in the multivalent groups segregate numerically evenly or unevenly. At anaphase chiasmata may still be present between some chromosomes, as seen in Fig. 4. This connection may still be preserved in some cases during the second division, as indicated in Fig. 5 (cf. VAARAMA, 1947).

Quite commonly some chromosomes are eliminated during the first division. In Fig. 4, for instance, a lagging bivalent can be observed, which most probably will later on be eliminated. Not infrequently a

whole chromosome group may in similar manner be left outside the spindle. The same is true of one or more univalents (cf. Fig. 5).

The second division is fairly regular. A few chromosomes are, however, left lagging (Fig. 6). They may be univalents divided already at first division and therefore no longer able to divide a second time. In other instances the division of a few univalents occurs first at the second division, and is also then delayed (Fig. 6). It seems probable that the chiasmata which were not broken normally at the first division are liable to hinder the normal anaphase separation of the chromosomes at the second division.

Owing to the uneven distribution of the chromosomes of the multivalent associations at anaphase I and to other meiotic irregularities the chromosome number in pollen grains varies considerably. The numbers of units in different metaphase plates at the second division has been found to be 26—35. Disturbances during the second division may still alter these numerical relationships.

The formation of pollen tetrads is, irrespective of what has been stated above, regular in its appearance. The chromosome eliminations do not result in a formation of micro-nuclei and micro-grains. In spite of the variable chromosome numbers in the four nuclei formed, the size of the mature pollen fluctuates but little. The average diameter, measured on 50 pollen grains, gave the value 35,06 $\mu$. Table 4 gives the distribution of the pollen in the different size-classes, using a grouping interval of 5 $\mu$. The corresponding values in a diploid have been obtained from the apple variety Sävstaholm, which variety according to HEILBORN (1928) possesses the diploid chromosome number $2n = 34$. In this variety the average pollen diameter 30,46 $\mu$ was measured. The size of the pollen grains in the tetraploid Hibernal is thus, in accordance with that found by JOHANSSON (1944), clearly larger.

TABLE 4. *Distribution of the pollen grains into different size-classes.*

| Size-class | 26—30 $\mu$ | 31—35 $\mu$ | 36—40 $\mu$ | 41—45 $\mu$ | 46—50 $\mu$ |
|---|---|---|---|---|---|
| Number of grains | | | | | |
| Hibernal ..... | 7 | 21 | 18 | 3 | 1 |
| Sävstaholm ..... | 28 | 21 | 1 | — | — |

The germination rate of the pollen of the Hibernal variety was 49,6 per cent (the value obtained in 20 hours with pollen kept in 15 per cent sucrose solution). The value found, however, is evidently

too low (cf. JOHANSSON, l. c.). The hard and snowless winter of 1946—47 caused many injuries to the root system of the apple trees, and apparently the reproductive organs of the flowers have also suffered, as was manifested in the malformation of their flowers.

## 2. SIZE OF THE STOMATA AND THE THICKNESS OF THE LEAVES.

Knowledge of the size of the stomata and the thickness of the leaves is of much value in connection with the establishing of the tetraploidy. To get an idea of the properties of the variety Hibernal in this respect measurements of these characters were made. Here, too, the variety Sävstaholm grown at the same place was used for comparison. Values for these characters in tetraploid apples are also to be found in several Swedish papers (JOHANSSON, 1937; ASCHÁN, 1943; NILSSON and LARSSON, 1944; OLDÉN, 1945, 1946).

The average measures for the stomata of the variety Hibernal seem to be as follows: length 31,1 $\mu$ and breadth 26,3 $\mu$, the index of breadth to length being 0,79. The corresponding values in the variety Sävstaholm are at the same time: length 27,4 $\mu$, breadth 20,7 $\mu$ and the b/l index 0,73. Thus, the stomata in the tetraploid are clearly larger than those in the diploid. The b/l index is also higher in the tetraploid, showing that the breadth of the stomata cells has here increased relatively more than the length. I have found the same also to be the case in the colchicine-induced tetraploid *Ribes nigrum* (VAARAMA, 1947). It seems that the difference in the index values between the diploids and the tetraploids are of equal order here as in the case of the apples. In the Swedish investigations only the length of the stomata has been given, some examples of which are given below in microns.

| | Pentapl. | Tetrapl. | Tripl. | Dipl. |
|---|---|---|---|---|
| ASCHÁN (1943) ..... | — | 35,3 $\mu$ | 33,7 $\mu$ | 27,9 $\mu$ |
| NILSSON and LARSSON (1944) | — | 34,1 » | 31,3 » | 27,0 » |
| OLDÉN (1945) ..... | — | 36,6—39,3 » | 33,7—35,2 » | — |
| OLDÉN (1946) ..... | 41,5 $\mu$ | 32,8 » | — | — |

The values found apparently correspond rather well with those in my material of the diploid Sävstaholm as well as with that of the tetraploid Hibernal.

The increase in the thickness of the leaves in the tetraploid is also ocularly observable. Measurements made on ten leaves have given the following average values: Sävstaholm 0,18 mm, Hibernal 0,22 mm. The

greater thickness of the leaves of the tetraploid apple varieties has been pointed out earlier by JOHANSSON (1937). OLDÉN (1945), in his turn, has published the average values as 0,360 mm for the tetraploids and 0,164—0,257 mm for the diploids. In another connection he gives the measures 0,17 mm for the tetraploid leaves and 0,20 mm for the still thicker pentaploid leaves. The thickness of the leaves is thus evidently a property highly influenced by external factors. In any case, however, a clear difference in the thickness of the leaves between diploids on the one side and tetraploids on the other side is always to be found in my material as well as in the Swedish.

## IV. DISCUSSION.

The origin of the variety Hibernal is unknown in its details. There is, however, reason to suppose that it has developed from a spontaneously occurring seedling.

For a spontaneous occurrence of a tetraploid there are two possibilities. One mode of origin is that which has been used in the production of the artificial tetraploids, i. e. an unreduced triploid gamete is fertilized by a normal gamete. Another possibility is indicated by EINSET's (1945) statement that when two diploid apple forms are crossed a small number of tetraploid offspring may arise through somatic doubling.

Remembering that all triploid apple varieties so far known have been found to be climatically more or less non-resistant, we cannot suppose the parental forms of the especially hardy variety Hibernal to belong to these. Therefore, with a higher degree of probability we can suppose that the variety Hibernal has arisen at the crossing of two diploid varieties in the manner described by EINSET.

Another problem is whether on the basis of the pairing conditions of the chromosomes in Hibernal anything may be concluded in regard to the make-up of chromosome complements in the supposed parental forms and the homologies prevailing between the chromosomes. Without doubt this question is too complicated to be solved with certainty. However, when sifting the different possibilities we may find one that is in agreement with the observed data.

The attempt to construct a chromosome formula for Hibernal has been based on the theory of the secondary polyploidy in the tribe Pomoidae (DARLINGTON and MOFFETT, 1930; MOFFETT, 1931; HEILBORN, 1935), which implies that a basic chromosome set consists of two primary groups of 7 chromosomes together with three chromosomes of

the group occurring additionally. If in addition we assume that the tetraploidy is effected by somatic doubling, the four secondary balanced groups of 17 chromosomes in the tetraploid must be identical in pairs.

Of the various possibilities available, I have found the adjoining chromosome formula to have the closest correspondence with the pairing conditions in the variety Hibernal as presented in Table 1. The exponents of the letters in the formula indicate the homologies between the chromosomes.

| | | | |
|---|---|---|---|
| $A_1A_1A_1$ | $A_1A_1A_1$ | $A_1A_1A_2$ | $A_1A_1A_2$ |
| $B_1B_2B_1$ | $B_1B_2B_1$ | $B_1B_2B_2$ | $B_1B_2B_2$ |
| $C_1C_2C_1$ | $C_1C_2C_1$ | $C_1C_2C_2$ | $C_1C_2C_2$ |
| $D_1D_2$ | $D_1D_2$ | $D_1D_1$ | $D_1D_1$ |
| $E_1E_2$ | $E_1E_2$ | $E_1E_2$ | $E_1E_2$ |
| $F_1F_2$ | $F_1F_2$ | $F_1F_2$ | $F_1F_2$ |
| $G_1G_2$ | $G_1G_2$ | $G_1G_2$ | $G_1G_2$ |

If we suppose that the above-presented homologies exist between the chromosomes, the maximal chromosome pairing will be as follows: $1_X$, $5_{VI}$, $6_{IV}$ and $2_{II}$. The possibilities of other combinations of chromosome associations are very great. To simplify the matter I have considered only the variation of even chromosome associations. In addition it is presumed in this connection that bivalent and tetravalent formation is complete and constant. Only decavalents have been supposed to break down, forming the combinations VIII + II, VI + IV and IV + IV + II, and hexavalents to form one quadrivalent and one bivalent.

I have calculated the average frequency of different chromosome associations per gamete that result from the 24 combinations which the restrictions presented above allow, and have modified the values to correspond with the ones given in Table 1. The resulting average frequencies from the different associations are then:

| II | IV | VI | VIII | X |
|---|---|---|---|---|
| 5,01 | 9,28 | 2,76 | 0,25 | 0,25 |

When comparing these values with the actual numbers in Table 1, we must bear in mind that the occurrence of complete higher associations is rare. Hence the theoretical possibility of the occurrence of decavalents has not been realized in the present material. Another important fact is that the theoretically calculated total number of associations greater than four is on an average equal to the total number actually

found. Similarly, the theoretical frequency of quadrivalents ought to be about the same as the actual frequency of trivalents and tetravalents, though naturally we cannot suppose all trivalents to arise from incomplete tetravalents. As can be seen, the theoretically calculated frequency fulfils satisfactorily these conditions.

The theoretical number of bivalents, on the other hand, is too high. It is evident that a considerable part of the theoretical bivalents which would arise from incomplete formation of higher associations remain in reality as univalents. We need only to suppose that if the theoretically possible decavalent is replaced by an octovalent and a bivalent, and the latter again by two univalents, depending on a failure of the chromosomes to pair, the theoretical number of bivalents sinks to 4,51.

On the basis of what has been stated it may be concluded that the chromosome formula given above corresponds in a considerable degree with the actual homologies which prevail amongst the chromosomes of the variety Hibernal.

As has been described in the Swedish papers already referred to several times, the artificially raised tetraploid apples show a number of gigas characters which are known to characterize especially autopolyploid plants (cf. MÜNTZING, 1936). This is seen in that the leaves are thicker and more distinctly dentate, the stomata are bigger, and the fruits of larger size, having a thicker skin and coarser flesh. The gigas characters increase parallel with the increase of the chromosome number. Most of these characters are also to be found in the variety Hibernal in correspondence with the artificially raised tetraploid apples. Morphologically the variety is thus a typical autotetraploid. The chromosomal conditions, too, i. e. the tendency to form multivalents at meiosis, would in this case indicate an autopolyploid origin.

It is very doubtful whether the exceptional climatic hardiness of the variety Hibernal depends on its polyploidy. It is a known fact that in severe climate there are polyploid plants in abundance (cf. LÖVE and LÖVE, 1943). This is, however, a very generalized conclusion and made from too heterogeneous a material to be applied to special cases. Moreover, it is known that none of the identified triploid apple varieties actually belong to a climatically hardy group. It seems, thus, more probable that the hardiness here met with should depend upon a suitable gene combination derived from the parents. The question of the supposed hardiness of tetraploid apples can be solved only through continued studies of the numerous artificially raised tetraploid apple forms.

I am greatly indebted to the Director of the State Horticultural Institute at Piikkiö, Professor O. MEURMAN, who has given the initiative to this study and helped me with good advice and criticism during the preparation of the manuscript.

## SUMMARY.

(1) A study has been made of the meiosis of the spontaneously arisen tetraploid apple variety Hibernal (LINCOLN, 1939) commonly cultivated in the most unfavourable regions for apple cultivation in the U. S. A. and Canada. Further, some measurements have been carried out of the size of the stomata and pollen grains as well as of the thickness of the leaves.

(2) A characteristic feature of metaphase I is the abundant formation of multivalents. Tetravalents are most commonly found. Tri- and penta-valent associations are somewhat rarer. Hexavalents appear in somewhat greater number than bivalents. The number of univalents varies from 0 to 6. Most commonly 2 or 3 univalents are present.

(3) Amongst the tri- and quadri-valent configurations the types most commonly met with are those (types according to DARLINGTON, 1931) which are due to the formation of more than one chiasma in a single chromosome arm. The chiasma frequency is 1,89 half chiasmata per chromosome.

(4) Univalents are found lagging and dividing after the separation of the conjugated chromosomes has already occurred. Several of the different chromosome associations as well as the univalents may be quite frequently eliminated.

(5) Some chromosomes are also found during anaphase II outside the spindle in the surrounding plasm. The formation of tetrads is, however, not influenced by these outsiders and is thus seemingly regular. The chromosome number of the pollen grains varies from 26 to 35. The germination rate of the pollen is rather good.

(6) The size of the pollen and stomata is larger than in diploid and triploid apple varieties, but smaller than in the known pentaploid seedling plant. Similarly, the thickness of the leaves and the size and the structure of the fruits show gigas characters.

(7) It has been thought that the variety Hibernal has arisen spontaneously by somatic doubling after an open pollination between two unknown climatically hardy diploid varieties.

(8) An attempt has been made to construct a chromosome formula representing the hypothetical homologies between the different chromosomes of the variety Hibernal.

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# CYTOLOGICAL STUDIES OF EXTRA FRAGMENT CHROMOSOMES IN RYE

## IV. THE POSITION OF VARIOUS FRAGMENT TYPES IN SOMATIC PLATES

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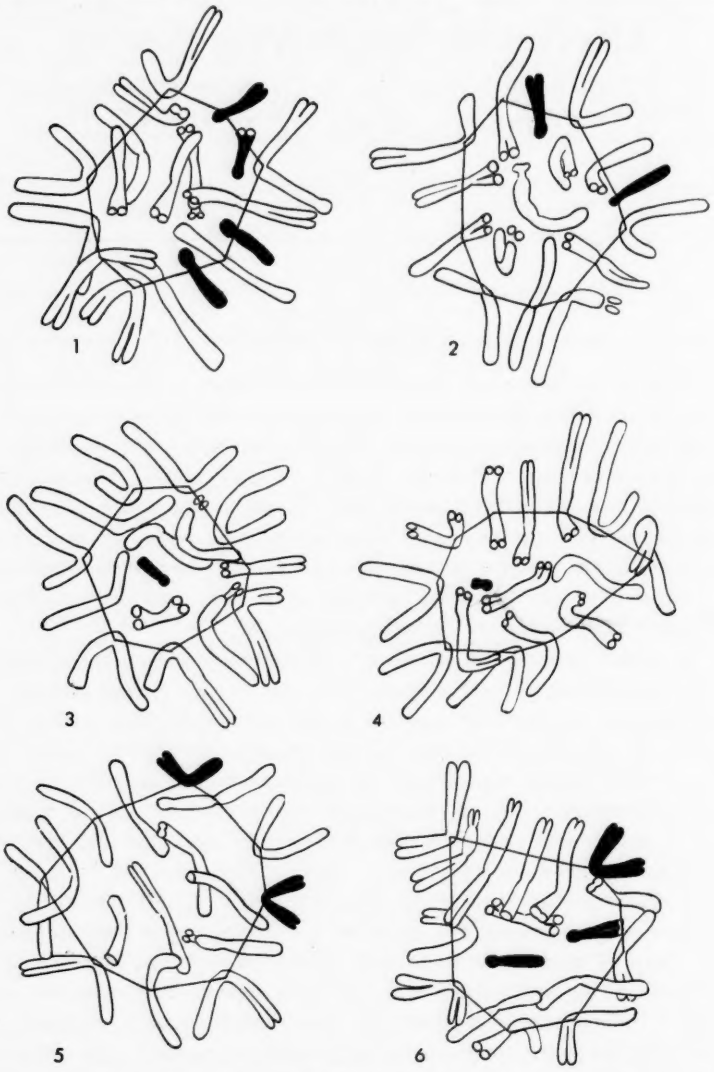
### I. INTRODUCTION.

IF somatic plates contain chromosomes of different size, it is a general experience that the smaller chromosomes tend to have a more central position than the larger ones. In *Zea mays* this rule is not valid for the B-chromosomes, which are present in some plants. According to DARLINGTON and UPCOTT (1941), these chromosomes, which are smaller than the normal chromosomes, have a tendency to be situated in the periphery of the somatic plates. This aberrant behaviour is considered to be caused by a weakness of the centromere, this weakness also resulting in a certain degree of somatic elimination.

In some respects the accessory chromosomes sometimes occurring in rye are similar to the B-chromosomes in maize. Hence, it might be interesting to study their position in the mitotic metaphase. During work with these peculiar extra chromosomes (MÜNTZING, 1943, 1944, 1945, 1946) a large number of chromosome counts in root tips have been undertaken. The corresponding drawings (for most of which I am much indebted to Mrs. E. LÖVKVIST) were available and represent the main material for the present study. This material includes the following types of accessory chromosomes (cf. MÜNTZING, 1944): (a) standard fragments, belonging to the two varieties Östgöta Gråråg and Vasa II and to hybrids and hybrid derivatives between these varieties, (b) small iso-fragments, probably representing twice the short arm of the standard fragment, (c) large iso-fragments, probably representing twice the long arm of the standard fragment, and (d) a new fragment type, similar to the standard fragment but with the long arm definitely shorter.

The different types of accessory chromosomes enumerated are present in the somatic plates represented by Figs. 1—6. These figures also illustrate the method used to indicate the position of the chromo-




Figs. 1—6. Somatic plates from root tips. B-chromosomes black, A-chromosomes in outline. — Fig. 1, Ög. $2n = 14 + 4$ standard fragments, position formula 8—6, 2—2; Fig. 2, Vasa $2n = 14 + 2$ standard fragments, position formula 9—5, 1—1; Fig. 3, Ög. $2n = 14 +$ new fragment type, position formula 8—6, 0—1; Fig. 4, Ög. $2n = 14 + 1$ small iso-fragment, position formula 10—4, 0—1; Fig. 5, Ög. $2n = 14 + 2$ large iso-fragments, position formula 7—7, 2—0; Fig. 6, $2n = 14 + 2$ s. f. $+ 1$ large iso-fragment, position formula 6—8, 0—2, 1—0. — × 3300.

somes in question. (For valuable suggestions concerning this method I am much indebted to Dr. G. ÖSTERGREN.) A line has been drawn connecting the centromeres of the chromosomes in the periphery. These chromosomes are chosen in such a way that the periphery line is never curved inward, i. e. the angle formed between the lines at both sides of a centromere is always 180° or greater when seen from the outside. By the periphery line the chromosomes are divided into two categories, the central ones with centromeres inside the periphery line and the peripheral chromosomes with centromeres situated at the line.

Fig. 1 represents a plate from an Östgöta Gråråg plant with $2n = 14 + 4$ standard fragments. Of the 14 normal chromosomes 8 are peripheral, 6 central. Two of the standard fragments are evidently peripheral, two central. Giving the peripheral chromosomes first, the formula for this plant will be 8—6, 2—2. Fig. 2 shows a plate from a Vasa II plant with $2n = 14 + 2$ standard fragments. The position formula of this plant is evidently 9—5, 1—1. The new fragment type, which deviates from the standard fragment by having the long arm diminished, is represented by Fig. 3. As this chromosome has a central position and 8 A-chromosomes are peripheral, the position formula will be 8—6, 0—1. Fig. 4 shows the small iso-fragment, the formula of this plate being 10—4, 0—1. The large iso-fragments are not always easy to distinguish from the smallest A-chromosomes. In Fig. 5, however, they may be identified, owing to their relatively small size and their strictly median centromere. The position formula of this plate is 7—7, 2—0. In Fig. 6, finally, there are two central standard fragments and one peripheral large iso-fragment in addition to the normal chromosomes, showing the distribution 6—8.

In a few of the normal chromosomes shown in Figs. 1—6 the position of the centromere is uncertain. In such cases the periphery line has been drawn to the middle of the chromosome. The error thus introduced is probably of no significance, as the normal rye chromosomes all have approximately median or submedian centromeres (cf. LEWITSKY, 1931; PATHAK, 1940; LEVAN, 1942). The same procedure was followed with regard to all the other drawings used for the analysis. With regard to the accessory chromosomes, however, it is necessary to know the exact position of the centromeres and, hence, only drawings in which all the centromeres of these chromosomes are clearly indicated were used for the calculations.

Before going into details, a few words may be said about the terminology used in this paper. For the sake of brevity the 14 normal

rye chromosomes are sometimes called A-chromosomes in contrast to the B-chromosomes, which represent the various types of extra fragment chromosomes occurring in the material studied. The denomination »extra fragment chromosome» is retained, as it was used in the title of the first paper in this series. Otherwise a more adequate name for the chromosome category in question is accessory chromosomes. Thus, B-chromosomes, extra fragment chromosomes and accessory chromosomes are synonymous denominations.

*Abbreviations*: Ög. or Östgöta=Östgöta Gråråg. — Vasa=Vasa II. — s. f. = standard fragment. — P = peripheral chromosomes. — C = central chromosomes. — % C = per cent central chromosomes.

## II. MEASUREMENTS.

### 1. CONTROL PLATES WITHOUT B-CHROMOSOMES.

In order to get information about the position of the normal rye chromosomes in somatic plates data were gathered from the following categories, the plants in each group being sister plants with $2n = 14$ of individuals carrying the different types of B-chromosomes indicated (Table 1).

TABLE 1. *Control plates without B-chromosomes.*

| Category | P | C | % C |
|---|---|---|---|
| Östgöta, s. f. ..... | 49 | 21 | 30,0 |
| Ög. × Vasa, 1946 ..... | 39 | 17 | 30,4 |
| » × » , 1947 ..... | 28 | 14 | 33,3 |
| Vasa × Ög. ..... | 139 | 57 | 29,1 |
| Ög., small iso-fragment ... | 446 | 184 | 29,2 |
| » , large » ... | 132 | 78 | 37,1 |
| » , new fragment type .. | 893 | 395 | 30,7 |
| Total | 1726 | 766 | 30,7 |

As is evident from the table, the percentage of central chromosomes ranges from 29,1 to 37,1 with an average of 30,7. A $\chi^2$-test was undertaken giving a $\chi^2$-value of 5,54 and a P intermediate between 0,50 and 0,30. Thus, there is no trace of heterogeneity.

With regard to the control plants, only one plate was generally drawn from each individual and, thus, the total of 178 plates represents 149 different plants. In material containing accessory chromosomes generally three plates per plant were examined, and in some cases a

larger number of plates per plant were studied. As no trace of a significant interplant or interplate variation was observed, all calculations in this paper are made with the chromosomes as units, the number of plates and plants not being specified.

Unfortunately the variety Vasa II is not represented in Table 1. This is because the mechanism of multiplication by means of non-disjunction at the first pollen mitosis is much more effective in Vasa II than in Östgöta Gråråg (cf. MÜNTZING, 1945). Hence, in the offspring of individuals with two standard fragments plants without such chromosomes are very seldom formed and were not available in the material studied.

## 2. PLATES WITH ONE STANDARD FRAGMENT ($2n = 14 + 1$ s. f.).

Data were available from both varieties and from hybrid derivatives of both reciprocal combinations. These hybrid derivatives had been produced in the following way: Plants of Östgöta Gråråg with $2n = 14 + 2$ large iso-fragments were pollinated with plants of Vasa II having $2n = 14 + 2$ standard fragments. In the offspring plants with $2n = 14 + 2$ standard fragments were intercrossed (in a crossing group). In the progenies of such plants some individuals with $2n = 14 + 1$ standard fragment were obtained. The somatic plates of such plants are represented in the category »Ög. × Vasa» in Table 2. In these plates the standard fragment is evidently derived from Vasa II, whereas the A-chromosomes represent a mixture from both varieties. In a similar way the category »Vasa × Ög.» in the table is derived, first from crosses between Vasa II plants with $2n = 14$ as female parents and plants of Östgöta Gråråg having $2n = 14 + 2$ standard fragments. Among the $F_1$ hybrids plants with $2n = 14 + 2$ s. f. were selected and intercrossed. In their progenies some plants had $2n = 14 + 1$ s. f. In these plants the standard fragment is evidently derived from Östgöta Gråråg, the A-chromosomes, on the contrary, representing a mixture from both varieties. The division of the A- and B-chromosomes into peripheral (P) and central (C) ones gave the following result (Table 2).

The first conclusion to be drawn from the table is that on an average the single standard fragment has a more central position than the other chromosomes. In the four categories of the table the percentage of central A-chromosomes ranges from 32,0 to 35,3, the corresponding values of the B-chromosomes ranging from 42,9 to 66,7. The grand total of all the plants with $14 + 1$ s. f. gives 32,8 per cent of central A-chromosomes and 59,9 per cent of central B-chromosomes.

TABLE 2. *Plates with 2n = 14 + 1 standard fragment.*

| Category | A-chromosomes | | | B-chromosomes | | | All chromosomes |
|---|---|---|---|---|---|---|---|
| | P | C | % C | P | C | % C | % C |
| Ög. ..... | 695 | 341 | 32,9 | 27 | 47 | 63,5 | 35,0 |
| Vasa × Ög. | 514 | 242 | 32,0 | 18 | 36 | 66,7 | 34,3 |
| Total | 1209 | 583 | 32,5 | 45 | 83 | 64,8 | 34,7 |
| Vasa ..... | 198 | 96 | 32,7 | 12 | 9 | 42,9 | 33,3 |
| Ög. × Vasa | 163 | 89 | 35,3 | 10 | 8 | 44,4 | 35,9 |
| Total | 361 | 185 | 33,9 | 22 | 17 | 43,5 | 34,5 |
| Grand total | 1570 | 768 | 32,8 | 67 | 100 | 59,9 | 34,7 |

The significance of this difference was controlled with a $\chi^2$-test, the $\chi^2$-value obtained being 50,3 and P much smaller than 0,001.

In the first two categories of the table the percentage of central B-chromosomes is about twice as high as that of the peripheral ones, in the last two categories, on the contrary, the central B-chromosomes are less numerous than the peripheral ones. This heterogeneity was tested with the $\chi^2$-method but was not found to be significant ($\chi^2 = 5{,}74$ and P for 3 degrees of freedom being intermediate between 0,20 and 0,10). However, there is reason to add the values of the first two categories, as the B-chromosome in Vasa × Östgöta is identical with the B-chromosome in Östgöta. In the same way, the B-chromosomes of the last two categories, Östgöta × Vasa and Vasa, are identical and the corresponding values may be added. This will result in the two distributions 45 : 83 and 22 : 17. A $\chi^2$-test of these values was undertaken, giving a $\chi^2$ of 5,62. This corresponds to a P-value intermediate between 0,02 and 0,01. Thus, it is highly probable that the standard fragment of Vasa II tends to have a more peripheral position than the corresponding chromosome of the other variety.

On account of this varietal difference it is necessary to reconsider the difference in position between the A- and B-chromosomes. As mentioned above, the grand total gave 59,9 per cent of central B-chromosomes, but the total material represents two rather different groups having the mean values 64,8 and 43,5. The former value represents the standard fragment of Östgöta Gråråg and was obtained from the ratio 45 : 83. The corresponding ratio of the A-chromosomes is 1209 : 583. A $\chi^2$-test showed that these distributions are significantly different, $\chi^2$ being 55,1 and $P < 0{,}001$. In the other category, Vasa + (Ög. × Vasa),

having 43,5 per cent of central B-chromosomes, the corresponding ratios are 22 : 17 and 361 : 185. In this case $\chi^2$ will be as low as 1,52 and P intermediate between 0,20 and 0,30. Thus, in plants with $2n = 14 + 1$ s. f. a significant difference in position between A- and B-chromosomes was only found with regard to the standard fragment of Östgöta Gråråg. However, a larger material of the other category will probably give a similar result.

### 3. PLATES WITH TWO STANDARD FRAGMENTS ($2n = 14 + 2$ s. f.).

Material of this kind was available from the same four categories as before, the following results being obtained (Table 3).

TABLE 3. *Plates with $2n = 14 + 2$ standard fragments.*

| Category | A-chromosomes | | | B-chromosomes | | | All chromosomes |
|---|---|---|---|---|---|---|---|
| | P | C | % C | P | C | % C | % C |
| Ög. ..... | 334 | 154 | 31,6 | 20 | 50 | 71,4 | 36,6 |
| Vasa × Ög. | 269 | 123 | 31,4 | 17 | 39 | 69,6 | 36,2 |
| Total | 603 | 277 | 31,5 | 37 | 89 | 70,6 | 36,4 |
| Vasa ..... | 104 | 64 | 38,1 | 12 | 12 | 50,0 | 39,6 |
| Ög. × Vasa | 686 | 338 | 33,0 | 54 | 102 | 65,4 | 37,3 |
| Total | 790 | 402 | 33,7 | 66 | 114 | 63,3 | 37,6 |
| Grand total | 1393 | 679 | 32,8 | 103 | 203 | 66,3 | 37,1 |

Again the standard fragments have a more central position than the normal chromosomes, the percentage values of the B-chromosomes being about twice as high as those of the A's. If the material is divided into the two categories corresponding to the standard fragments of Östgöta Gråråg and Vasa II, the distributions to be compared will be 603 : 277 and 37 : 89 in the first category and 790 : 402 and 66 : 114 in the second category. In both cases a statistical test gives quite high $\chi^2$-values, thus demonstrating that the difference in position between A- and B-chromosomes is significant not only in Östgöta Gråråg but also in Vasa II.

In the material with two standard fragments the differential behaviour between the standard fragments of Östgöta and Vasa may again be traced. In the pure varieties the percentage values of central B-chromosomes are 71,4 and 50,0 respectively. A $\chi^2$-test of the distributions 20 : 50 and 12 : 12 gives a $\chi^2$ of 3,66 and a P intermediate

between 0,10 and 0,05. If »Östgöta» and »Vasa × Östgöta» are added and the same summation is undertaken with »Vasa» and »Östgöta × Vasa» the distributions to compare will be 37 : 89 and 66 : 114. This gives a $\chi^2$ of 1,77 and a P intermediate between 0,20 and 0,10. The percentage values corresponding to these distributions are 70,6 and 63,3 respectively. Thus, though the differences tested are not significant, it is nevertheless more probable that the standard fragments of the two varieties behave differently than that they react in the same way.

### 4. PLATES WITH THREE OR FOUR STANDARD FRAGMENTS.

A few plates with $2n = 14 + 3$ s. f. were available and showed the following distributions (Table 4).

TABLE 4. *Plates with $2n = 14 + 3$ standard fragments.*

| Category | A-chromosomes | | | B-chromosomes | | | All chromosomes |
|---|---|---|---|---|---|---|---|
| | P | C | % C | P | C | % C | % C |
| Ög. ..... | 111 | 57 | 33,9 | 8 | 28 | 77,8 | 41,7 |
| Vasa × Ög. | 16 | 12 | 42,9 | 0 | 6 | — | 52,9 |
| Total | 127 | 69 | 35,2 | 8 | 34 | 81,0 | 43,3 |
| Vasa ..... | 34 | 8 | 19,0 | 4 | 5 | — | 25,5 |
| Ög. × Vasa | 19 | 9 | 32,1 | 2 | 4 | — | 38,2 |
| Total | 53 | 17 | 24,3 | 6 | 9 | 60,0 | 30,6 |
| Grand total | 180 | 86 | 32,3 | 14 | 43 | 75,4 | 39,9 |

The values are too scanty for statistical treatment, but the same tendency for the standard fragment of Vasa to have a more peripheral position than that of Östgöta may again be noticed. It is also obvious that the B-chromosomes are more central than the A-chromosomes.

A larger number of plates with $2n = 14 + 4$ s. f. was available, the following frequencies and percentage values being obtained (Table 5).

With regard to the relative position of the A- and B-chromosomes the general picture is evidently the same as before, and a statistical treatment is in this case superfluous. Comparing the behaviour of the standard fragments of Vasa and Östgöta, the conditions are more obscure. The highest percentage of central standard fragments was again found in Östgöta (80,9), the corresponding value in Vasa being ten per cent lower (70,8). However, a $\chi^2$-test of the distributions in question

TABLE 5. *Plates with 2n = 14 + 4 standard fragments.*

| Category | A-chromosomes | | | B-chromosomes | | | All chromosomes |
|---|---|---|---|---|---|---|---|
| | P | C | % C | P | C | % C | % C |
| Ög. ..... | 167 | 71 | 29,8 | 13 | 55 | 80,9 | 41,2 |
| Vasa × Ög. | 79 | 33 | 29,5 | 10 | 22 | 68,8 | 38,2 |
| Total | 246 | 104 | 29,7 | 23 | 77 | 77,0 | 40,2 |
| Vasa ..... | 163 | 89 | 35,3 | 21 | 51 | 70,8 | 43,2 |
| Ög. × Vasa | 240 | 152 | 38,8 | 26 | 86 | 76,8 | 47,2 |
| Total | 403 | 241 | 37,4 | 47 | 137 | 74,5 | 45,7 |
| Grand total | 649 | 345 | 34,7 | 70 | 214 | 75,4 | 43,7 |

(13 : 55 and 21 : 51) gave a $\chi^2$-value of 1,92 and a P intermediate between 0,20 and 0,10. Adding the first two categories of the table, the average percentage of central standard fragments derived from Östgöta Gråråg will be 77,0. The corresponding value of the other variety (obtained by adding the values of Vasa and Östgöta × Vasa) will be 74,5. Thus, though the average value representing the Vasa fragment is again lower than that of Östgöta, the difference is not significant.

Interesting enough, there is a clear difference between the A-chromosomes of these categories. As may be seen from the table, the percentage of central A-chromosomes is 29,8 in Östgöta and 29,5 in Vasa × Östgöta, the total average for these two categories being 29,7. In the two categories representing the Vasa fragment the percentages of central A-chromosomes are 35,3 and 38,8 with an average of 37,4. The distributions underlying the percentages 29,7 and 37,4 are 246 : 104 and 403 : 241 respectively. A $\chi^2$-test of these figures gives a $\chi^2$-value of 5,94 and a P intermediate between 0,02 and 0,01. Thus, it is highly probable that the A-chromosomes are distributed differently in the two categories. The large proportion of central A-chromosomes in the Vasa category is probably correlated to the small proportion of central B-chromosomes. In the Östgöta category, on the contrary, the inverse situation is met with. There is a smaller proportion of central A-chromosomes and a larger proportion of central B-chromosomes.

The occurrence of a significant difference between the A-chromosomes and the non-significance with regard to the B-chromosomes might be due to two different causes. One possibility would be that the central B-chromosomes in Vasa though situated within the periphery line are closer to this line than the B-chromosomes in the other variety.

This possibility was tested by measurements of the average distance between the B-chromosomes and the periphery line and by expressing this distance in per cent of the average diameter of the plate. These measurements revealed no difference between Vasa II and Östgöta Gråråg and, hence, it is superfluous to report the detailed data leading to this conclusion.

The other possible explanation is simply that the A-chromosomes are 3,5 times as numerous as the B-chromosomes and, therefore, if there is a significant varietal difference with regard to A- as well as B-chromosomes, the level of significance will first be attained by the more numerous A-chromosomes. In other words, the difference now indicated between the B-chromosomes will probably reach significance if the material is sufficiently increased.

This argumentation is to some extent supported by the data from plates with 14 + 2 s. f. In these cases the proportion of central A-chromosomes is again somewhat higher in material with the Vasa fragment than in material with the Östgöta fragment. However, the difference between the percentage values (33,7 and 31,5) is not significant. It is to be expected, however, that a noticeable change in the position of the A-chromosomes will only be obtained if the number of B-chromosomes is sufficiently high. The influence is strong enough to be clearly demonstrated in plants with 4 B-chromosomes but is less pronounced in plants with 2 B-chromosomes. A trace of the same phenomenon is even indicated in plants with only one B-chromosome (Table 2).

### 5. COMPARISON BETWEEN THE CLASSES WITH DIFFERENT NUMBERS OF STANDARD FRAGMENTS.

The percentages of central A- and B-chromosomes in plates with different numbers of B-chromosomes have been brought together in Table 6. In this table the data for each variety or hybrid progeny is given as well as the total values. Percentage values were not calculated when the number of variates is lower than 25. If this number is higher than 24 but lower than 50 the corresponding percentage value is put in brackets. In all other cases the number of variates is higher than 49. The actual numbers may be found in Tables 1—5.

The grand total for the entire material in each chromosome number class shows a steady increase in the percentage of central chromosomes. In the five classes 14, 14 + 1, 14 + 2, 14 + 3 and 14 + 4 the percentage values are 29,9, 34,7, 37,1, 39,9 and 43,7 respectively. This increase is a direct consequence of the increase in absolute chromosome number.

TABLE 6. *Percentage of central A- and B-chromosomes in plates with 0—4 standard fragments.*

| Category | 2n = 14 | 2n = 14 + + 1 s.f. | 2n = 14 + + 2 s.f. | 2n = 14 + + 3 s.f. | 2n = 14 + + 4 s.f. |
|---|---|---|---|---|---|
| Ög., A | 30,0 | 32,9 | 31,6 | 33,9 | 29,8 |
| » , B | — | 63,5 | 71,4 | (77,8) | 80,9 |
| » , A + B | 30,0 | 35,0 | 36,6 | 41,7 | 41,2 |
| Vasa × Ög., A | 29,1 | 32,0 | 31,4 | (42,9) | 29,5 |
| » × » , B | — | 66,7 | 69,6 | — | (68,8) |
| » × » , A + B | 29,1 | 34,3 | 36,2 | 52,9 | 38,2 |
| Ög. + (Vasa × Ög.), A | 29,3 | 32,5 | 31,5 | 35,2 | 29,7 |
| » + ( » × » ), B | — | 64,8 | 70,6 | (81,0) | 77,0 |
| » + ( » × » ), A + B | 29,3 | 34,7 | 36,4 | 43,3 | 40,2 |
| Vasa, A | — | 32,7 | 38,1 | (19,0) | 35,3 |
| » , B | — | — | — | — | 70,8 |
| » , A + B | — | 33,3 | 39,6 | 25,5 | 43,2 |
| Ög. × Vasa, A | 31,6 | 35,3 | 33,0 | (32,1) | 38,8 |
| » × » , B | — | — | 65,4 | — | 76,8 |
| » × » , A + B | 31,6 | 35,9 | 37,3 | (38,2) | 47,2 |
| Vasa + (Ög. × Vasa), A | 31,6 | 33,9 | 33,7 | 24,3 | 37,4 |
| » + ( » × » ), B | — | (43,5) | 63,3 | — | 74,5 |
| » + ( » × » ), A + B | 31,6 | 34,5 | 37,6 | 30,6 | 45,7 |
| Total, A | 29,9 | 32,8 | 32,8 | 32,3 | 34,7 |
| » , B | — | 59,9 | 66,3 | 75,4 | 75,4 |
| » , A + B | 29,9 | 34,7 | 37,1 | 39,9 | 43,7 |

When the number is high, the surface of the chromosome plate will be larger in relation to the periphery than when the number is low and the plate smaller.

With regard to this total increase the A- and B-chromosomes behave differently. As may be seen from the table, the increase in the percentage of central A-chromosomes is very slight, the series of A-values being 29,9, 32,8, 32,8, 32,3 and 34,7. The proportion of central B-chromosomes, on the contrary, increases markedly on passing from 14 + 1 to 14 + 4. In this case the series is 59,9, 66,3, 75,4, 75,4. The third value (for 14 + 3) is less exact than the other three, being based on only 57 variates. Hence, the increase from 14 + 1 to 14 + 4 is probably continuous. At any rate, the data are sufficient to show that when the absolute chromosome number increases it is chiefly the B-chromosomes which take up a central position. Thus, the different position of the

A- and B-chromosomes in somatic plates is most marked when the number of B's is high.

The data in Table 6 may be used for constructing the diagram in Fig. 7, which clearly shows the differential behaviour of the standard fragments of the two varieties Östgöta Gråråg and Vasa II. In the

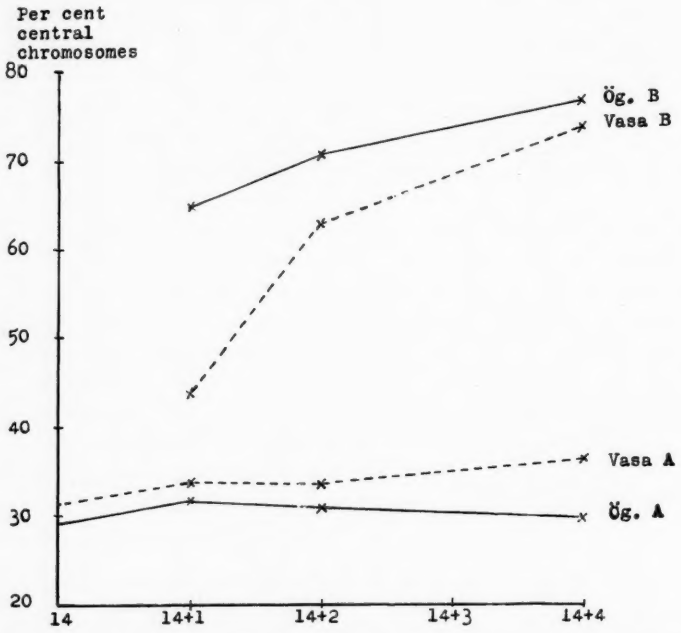



Fig. 7. Percentage of central standard fragments (B) and normal chromosomes (A) in somatic plates of two rye varieties and their hybrid derivatives. The categories Ög. A and B also include material from the cross Vasa × Ög. In the same way the categories Vasa A and B also include material from the cross Ög. × Vasa.

curves the values of the class 14 + 3 have been omitted, these values being too uncertain on account of the low number of variates.

From the diagram it is clear that in both varieties the B-chromosomes have a more central position than the A-chromosomes and that the percentage of central B-chromosomes shows a marked increase as the absolute number of B-chromosomes increases. However, the differential behaviour of the two chromosome categories is more marked with regard to the B-chromosomes of Östgöta than with regard to those of Vasa. In Östgöta + (Vasa × Ög.) the curve representing the percentage

of central B-chromosomes is for all chromosome number classes situated above the corresponding curve of Vasa + (Ög. × Vasa). The difference between the curves ist most pronounced when the number of B-chromosomes is low. When the number is high, most of the B-chromosomes become central even in the Vasa material. With regard to the A-chromosomes the conditions are just reversed. In all chromosome number classes the Vasa material shows a higher percentage of central A-chromosomes than the material carrying the standard fragment of Östgöta Gråråg. The difference ist most pronounced when the absolute chromosome number is high. As suggested above, this is probably so because a noticeable change in the position of the A-chromosomes can only occur when the number of B-chromosomes is sufficiently high.

## 6. PLATES WITH B-CHROMOSOMES SMALLER THAN THE STANDARD FRAGMENT.

This group of material comprises two categories, viz. the small iso-fragment (Fig. 4), representing twice the short arm of the standard fragment and, secondly, the new fragment type (Fig. 3) which is similar to the standard fragment but in which the long arm is shortened. This new fragment type has lost the ability of numerical increase by non-disjunction and the same is true with regard to the small iso-fragment. Thus, only plates with $2n = 14 + 1$ fragment were available for study in these two categories. Both these fragment types belong to the variety Östgöta Gråråg. The following data were obtained (Table 7).

TABLE 7. *Plates with small B-chromosomes.*

| Category | A-chromosomes | | | B-chromosomes | | | All chromosomes |
|---|---|---|---|---|---|---|---|
| | P | C | % C | P | C | % C | % C |
| 14 + 1 small iso-fragment | 747 | 303 | 28,9 | 21 | 54 | 72,0 | 31,7 |
| 14 + 1, new fragment type | 1127 | 469 | 29,4 | 39 | 75 | 65,8 | 31,8 |

From the table it is evident that in both categories the B-chromosomes have a more central position than the other chromosomes. A $\chi^2$-analysis of the distributions 1127 : 469 and 39 : 75 gives a $\chi^2$-value of 65,0 and a P smaller than 0,001. The same test of the distributions 747 : 303 and 21 : 54, which represent the small iso-fragment, gave a $\chi^2$ of 60,1 and a P smaller than 0,001.

The percentage values of central B-chromosomes were in these cases 65,8 (new fragment type) and 72,0 (small iso-fragment). These values are both higher than the percentage of central B-chromosomes

in plants of Östgöta Gråråg with $2n = 14 + 1$ standard fragment, this value being 63,5 (Table 2). The corresponding percentage in Ög. + (Vasa × Ög.) is 64,8. Thus, comparing standard fragment, new fragment type and small iso-fragment, there seems to be a negative correlation between size and degree of central position. This would be in good accordance with the relations between the normal chromosomes and the standard fragments. However, the data available are not large enough to be statistically significant, a $\chi^2$-test showing no significant heterogeneity. Thus, so far, it may only be concluded that the smallest B-chromosome types show about the same degree of central position as the standard fragment, and like this B-chromosome type they are significantly more central than the A-chromosomes.

## 7. PLATES WITH LARGE ISO-FRAGMENTS.

In contrast to the previous types of B-chromosomes the large iso-fragment is sometimes difficult to distinguish in somatic plates. It is almost as large as the smallest A-chromosomes and does not differ conspicuously from the A-chromosomes with regard to the position of the centromere. In good plates, however, the large iso-fragments may be recognized rather easily, being distinguished from the A-chromosomes by their somewhat smaller size and the exactly median centromere. The data given below are gathered from a limited number of plates controlled by me, in which the large iso-fragments could be identified with certainty or with a high degree of probability. Considering first the plates containing no other types of B-chromosomes than large iso-fragments the following data are available (Table 8).

TABLE 8. *Plates with large iso-fragments.*

| Category | A-chromosomes | | | B-chromosomes | | | All chromosomes |
|---|---|---|---|---|---|---|---|
| | P | C | % C | P | C | % C | % C |
| 1 large iso-f., Vasa × Ög. .... | 28 | 14 | 33,3 | 3 | 0 | — | 31,1 |
| 2 » » , Ög. ..... | 207 | 115 | 35,7 | 32 | 14 | 30,4 | 35,1 |
| 2 » » , » × Vasa .... | 37 | 19 | 33,9 | 6 | 2 | — | 32,8 |
| 3 » » , » ..... | 19 | 9 | — | 2 | 4 | — | 38,2 |
| 4 » » , » ..... | 40 | 30 | 42,9 | 14 | 6 | 30,0 | 40,0 |
| Total | 331 | 187 | 36,1 | 57 | 26 | 31,3 | |

From these data it is obvious that the large iso-fragment has a more peripheral position than all the other types of B-chromosomes studied. To prove this statement it is sufficient to compare the position of the

B-chromosomes in the categories Ög. 14 + 2 large iso-fragments and Ög. 14 + 2 standard fragments (Table 3). The distributions in question are 32 : 14 and 20 : 50 respectively. A $\chi^2$-test gave a $\chi^2$-value of 18,9 which corresponds to a P smaller than 0,001.

The second difference to be considered is that between the large iso-fragments and the normal chromosomes. The large iso-fragments seem to have a more peripheral position than the A-chromosomes, but the data are not sufficient to prove that definitely. If all values from plants with large iso-fragments are added to one series the A-chromosomes will have the ratio 331 : 187, which corresponds to 36,1 per cent of central A-chromosomes. The corresponding values of the large iso-fragments are 57 : 26 and 31,3 per cent. A $\chi^2$-test showed that this difference is not significant. It should also be kept in mind that the identification of the large iso-fragments is not absolutely reliable. As the peripheral chromosomes are generally easier to distinguish than the central ones, errors in classification will tend to increase the calculated percentage of peripheral large iso-fragments. Thus, at present it can only be concluded that the position of the large iso-fragments is about the same as that of the A-chromosomes.

In some cases the plates available contained large iso-fragments as well as standard fragments. In such plates the large iso-fragments had a clear tendency to be more peripheral than the standard fragments (Fig. 6). However, so far, the number of clear plates of this kind is too small to allow a statistical analysis.

## III. DISCUSSION.

A special arrangement of chromosomes of different size at the mitotic metaphase is of interest in connection with attempts to explain the mechanism of mitosis. If small chromosomes have another position in the plates than large chromosomes, this must certainly be a reflexion of forces involved in the complicated process of mitosis. The more central position of small chromosomes in somatic plates is a wellknown phenomenon reported by many cytologists. A number of such cases are cited by TISCHLER (1942, p. 183). It is generally assumed that the arrangement in question has some connection with the viscosity of the protoplasm, the small chromosomes moving more freely than the large ones.

ÖSTERGREN (1945) has considered the problem and concludes that the position of a chromosome in the plate is determined by two

antagonistic tendencies, the centromeres striving to move inward (centripetally) and the chromatids to move outward (centrifugally). The centrifugal force arises because the spindle is probably a tactoid-like structure characterized by a definite spatial arrangement of mobile particles in a dynamic equilibrium. Hence, the presence of any body inside the spindle modifying the spontaneous equilibrium position of the particles will increase the potential energy of the tactoid. Thus, the tactoid will strive to remove disturbing bodies like chromosomes. This will occur in a transverse direction on account of the simultaneous longitudinal forces between poles and centromeres. According to this theory, small chromosomes will tend to have a central position because they are less disturbing to the spindle than larger chromosomes.

The results obtained in the present investigation clearly show a grouping according to size. The normal chromosomes are larger and more peripheral than the standard fragment, which is about half as large as the A-chromosomes. Of the various new chromosome types derived from the standard fragment, those which are smaller (the small iso-fragment and a new type which has lost part of the long arm) have the same position as the standard fragment or are still more central. The large iso-fragment, on the contrary, which is almost twice as large as the standard fragment, is significantly more peripheral than the standard fragment and the small fragment types. The large iso-fragment is about as large as the smallest A-chromosomes and was not found to differ significantly in position from the average of the A-chromosomes.

The starting-point of this investigation was to determine whether the standard fragments in rye had the same tendency to a peripheral position as the B-chromosomes in maize (DARLINGTON and UPCOTT, 1941). The results show that this is not the case, the standard fragments in rye, on the contrary, being more central than the normal chromosomes. If a small chromosome is characterized by a peripheral position, this indicates that the centromere reacts in another way than the centromeres of the other chromosomes, and this deviation may be described as a weakness of the centromere. The standard fragment in rye evidently does not show any weakness of this kind but seems to have a centromere of normal strength. This is in good accordance with the fact that this chromosome is very seldom eliminated in the somatic mitosis, the same number of standard fragments being present in root tips and at meiosis in the p.m.c. In maize, on the contrary, the B-chromosomes are often eliminated (l. c.). Another similar case, in

which frequent elimination is accompanied by a peripheral position at mitosis, is probably represented by a diploid sexual strain of *Poa alpina* (MÜNTZING, 1946 b, 1948; HÅKANSSON, 1948). However, the preliminary observations made in this material need further verification.

In *Secale* the derivatives of the standard fragment are of special interest. This implies that from a definite chromosome type, which may easily be recognized at mitosis, new and deviating chromosome types have arisen, the position of which in somatic plates may also be determined. Besides, these new types have arisen by misdivision of the standard fragment, and this might be expected to influence the strength of the centromeres in the products of misdivision (DARLINGTON, 1939, 1940; cf. also MÜNTZING, 1946 a, pp. 114—115).

As far as the present observations go, the small and large iso-fragments seem to have the same centromere strength as the original chromosome, the standard fragment. The large iso-fragment has a more peripheral position than the standard fragment, but this is more likely due to the larger size than to a weakening of the centromere. The centromere of the small iso-chromosome also seems to have retained its strength, this chromosome having the same or a still more central position than the standard fragment. Though not yet conclusive these observations from the somatic mitosis as well as previous observations from meiosis and pollen mitosis (MÜNTZING, 1944, 1946) indicate that the process of misdivision in these cases does not change the strength of the centromere. This is in agreement with recent observations by ÖSTERGREN (1947), who finds that the actively mobile component of the centromere at mitosis is divided into two parts (»spindle spherules») corresponding to the two chromatids not only at metaphase but also at early prophase. Furthermore, each spindle spherule is in reality double, and in cases of misdivision the plane of division will separate these half spherules. Later on this may result in iso-chromosomes, the centromere apparatus of which is probably identical with that of the original chromosome.

The arrangement of chromosomes according to size in somatic metaphases seems to be a phenomenon of general occurrence in plants as well as animals. This indicates that, as a rule, chromosomes of different sizes have centromeres of the same strength. These centromeres may even be identical. However, exceptional cases such as the B-chromosomes in maize and the iso-chromosome in *Nicandra* (DARLINGTON and JANAKI-AMMAL, 1945) demonstrate that the strength of reaction of the centromeres is not always the same for all chromosomes in a

metaphase plate. Thus, in such cases the arrangement of the chromosomes is not exclusively conditioned by their size.

An unexpected result of the present investigation is the demonstration of a significant difference between the two rye varieties Östgöta Gråråg and Vasa II with regard to the position of the standard fragments. In the former variety these chromosomes have a more central position than in the latter variety, the differential behaviour of normal chromosomes and standard fragments thus being more pronounced in Östgöta Gråråg than in Vasa II (Fig. 7).

This difference is most pronounced in plates with one standard fragment. When the absolute number of chromosomes becomes higher, the proportion of central chromosomes will also increase. This increase will predominantly involve the standard fragments, whereas the proportion of central A-chromosomes remains almost constant. Thus, in plants with several standard fragments these chromosomes have a marked tendency to occupy the central area in the plate. This tendency is obvious in both rye varieties and tends to obliterate the differential behaviour observed when the number of standard fragments is low.

The standard fragments of these rye varieties have been observed to be different also in other respects. Thus, in plants with two standard fragments these chromosomes are almost regularly paired at meiosis in Vasa II, whereas in Östgöta Gråråg there is a high degree of non-conjunction. The exact figures are 89 per cent of the p.m.c. with paired standard fragments in Vasa II, the corresponding value in Östgöta Gråråg being as low as 38 per cent (MÜNTZING, 1945, p. 469).

There is also a slight difference in size between the standard fragments of Östgöta and Vasa. In root tips the Vasa fragment was found to be 11,5 per cent longer than the Östgöta fragment and a similar difference was also observed at meiosis (MÜNTZING, 1945, p. 469). A less clear difference was observed at the first pollen mitosis. At this critical stage, characterized by a directed non-disjunction of the standard fragments in both varieties, the fragment of Vasa II seems to reach the generative pole earlier than in Östgöta Gråråg and does not lag so much at the equator as in the latter variety (MÜNTZING, 1946 a, p. 105).

Whether there is any causal connection between these slight differences, earlier observed, and the difference in position in somatic plates is at present obscure. It does not seem probable that the small difference in size between the standard fragments of the two varieties is sufficient to explain the different position in the plates. Some other cause is probably responsible, and this cause might *a priori* be looked

for either in the general genotype of the varieties or in the structural or genotypical properties of the standard fragments themselves. The present results are rather in favour of the latter possibility. As may be seen from Tables 2—6, there is an obvious tendency for the hybrids Vasa × Östgöta to behave in the same way as pure Östgöta and for the hybrids Östgöta × Vasa to give similar values to pure Vasa. In other words, the standard fragments of the two varieties seem to retain their characteristic properties also when present in a quite new and hybrid genotypical environment. The present data are not sufficient to prove that definitely. More evidence may be obtained from an analysis of size and degree of meiotic pairing of the standard fragments in reciprocal hybrids between the two varieties.

## SUMMARY.

(1) Various types of accessory chromosomes in rye were studied with regard to their position in somatic metaphase plates. By drawing a periphery line the chromosomes in such plates were divided into two categories, the central ones with centromeres inside the periphery line and the peripheral chromosomes with centromeres situated at the line. The percentage of central chromosomes was determined for the normal chromosomes, for standard fragments and for derivatives of the standard fragment (large and small iso-fragments and a new type in which the long arm of the standard fragment is diminished).

(2) The chromosomes were found to be arranged in order of size, the smallest types being most central. Thus, in contrast to the behaviour of the B-chromosomes in maize, the standard fragments in rye are more central than the normal chromosomes. Judging from their position the large and small iso-fragments, derived from the standard fragment, have normal centromeres of the same strength as in the standard fragment and the normal chromosomes.

(3) The two rye varieties Östgöta Gråråg and Vasa II differ significantly with regard to the behaviour of their standard fragment. In Östgöta Gråråg this chromosome has, on an average, a more central position than in the other variety. As this differential behaviour also occurs in hybrids between the two varieties, the difference in position is probably caused by structural or genotypical differences inherent in the fragments.

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# STUDIES ON THE EXPERIMENTAL CONTROL OF THE MUTATION PROCESS

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## THE PROBLEM.

THE mutation work at the Swedish Seed Association proceeds along three lines: (1) Vital mutants are induced and isolated in different agricultural species, especially in cereals, and their practical value is examined. (2) The viability reaction of spontaneous and induced mutations — vital as well as lethal — is tested by and by in segregating progenies, both with regard to the heterozygous and the homozygous phase. (3) Extensive experiments aim at an intentional control and direction of the mutation process.

With special regard to the third problem, which is the one of interest here, some results have already been published (GUSTAFSSON, 1940, 1946 a and b, 1947). They indicate that a pre-treatment of the seeds before irradiation influences not only the rates of mutation, but furthermore the types of mutation appearing. Chlorophyll mutations, frequent and characteristic, serve as an excellent model material. The large material required and the immense labour involved limit the range of the results considerably. The accumulation of new data, year after year, should however help to make some definite conclusions possible. In this paper some further facts will be presented.

## MATERIAL.

In our experiments we used seeds of a high-yielding barley strain (01513 $b_7$), a sister-line to the so-called Ymer variety. Since small and large seeds react somewhat differently to the irradiation, seeds of a distinct size (from the largest sieve of 2,8 mm.) were pre-treated with different chemicals and then irradiated with 7,500 r-units. The water content was approximately 11 %. In order to exclude all spontaneous incrossings and contaminations the $X_1$ generation was grown surrounded by oats and spring wheat on all sides.

The chemicals included four colchicine solutions (0,1, 0,05, 0,01 and 0,005 %), potassium cyanide (0,01, 0,001 and 0,0001 molar solution), hydro-

gen peroxide (1 and 0,5 %), butter-yellow (saturated solution), uranyl nitrate (0,001 and 0,0001 mol.), ferri sulphate (0,1 mol.), distilled water, as well as buffer solutions with a pH of 3 and a pH of 10. The seeds were enveloped in filter paper, moistened with a certain amount of the different solutions, and then kept in petri dishes for 24 hours at a room temperature of ca. 15 centigrades. Afterwards the seeds were washed in tap water and dried off between filter papers. Irradiation was carried out 24 hours later in the Radiophysics Institute, Stockholm, under the supervision of Dr. A. FORSSBERG and in the manner described by the senior author in previous papers.

The types and rates of mutation were determined in laboratory experiments (completed with some field analyses), performed in such a way that an equal number of spikes from the various series were laid out repeatedly until the gathered material allowed some definite conclusions.

The pre-treatments were carried out at the Chromosome Department of the Swedish Seed Association by GUSTAFSSON in collaboration with Dr. A. LEVAN (April, 1946). The mutation analyses were performed by D'AMATO and GUSTAFSSON, with the assistance of Mr. C. G. VON SYDOW. We are indebted to Professor A. MÜNTZING for financial support.

## RESULTS.

*The $X_1$ effect* (Table 1). — The following properties were determined, (1) the percentage of ripe $X_1$ plants, (2) the average fertility of the $X_1$ plants, (3) the percentage of normally fertile $X_1$ plants (possessing less than 10 % empty spikelets, i. e. a fertility of 90—100 %).

In general, the pre-treated series showed a more intense irradiation effect than the dry series.

*The series treated with KCN* were markedly influenced with regard to all three properties. The highest concentration was more effective than, or at least equally effective as, the ten times diluted solution, in spite of the low production of mutations.

In none of the concentrations used did the *colchicine solutions* decrease the $X_1$ fertility and the number of fully fertile $X_1$ plants. Under this pre-treatment, however, the seedling lethality was more pronounced than in the dry series.

Uranyl nitrate and butter-yellow influenced the $X_1$ generation profoundly, especially with regard to plant fertility. The number of ripe $X_1$ plants was surprisingly high in the concentrated solution of

TABLE 1. *The combined effect of chemical pre-treatment and irradiation (7.500 r). The $X_1$ generation.*

| Pre-treatment | Number of mature plants | Average plant fertility | Number of normally fertile plants | Examined $X_1$ plants |
|---|---|---|---|---|
| Control series | — | 97,2 % | 96,7 % | 30 |
| Dry seed series | 86 % | 84,4 | 33,6 | 101 |
| Colchicine 0,1 % | 75 | 82,3 | 31,3 | 99 |
| » 0,05 » | 78 | 86,4 | 47,0 | 100 |
| » 0,01 » | 67 | 86,1 | 36,0 | 50 |
| » 0,005 » | 74 | 83,3 | 36,0 | 50 |
| Potassium cyanide (KCN) 0,01 mol. | 29 | 71,7 | 13,8 | 87 |
| » » » 0,001 » | 24 | 73,2 | 14,1 | 71 |
| » » » 0,0001 » | 55 | 79,8 | 22,9 | 48 |
| Uranyl nitrate 0,001 mol. | 89 | 74,9 | 6,0 | 100 |
| » » 0,0001 » | 38 | 76,9 | 7,1 | 99 |
| Hydrogen peroxide ($H_2O_2$) 1 % | 33 | 83,2 | 36,7 | 98 |
| » » » 0,5 » | 52 | 79,8 | 34,3 | 102 |
| Butter-yellow, saturated | 68 | 72,9 | 15,0 | 100 |
| $Fe_2(SO_4)_3$ 0,1 mol. | 42 | 83,6 | 33,0 | 100 |
| pH = 3 | 70 | 80,3 | 26,0 | 100 |
| pH = 10 | 65 | 85,3 | 33,3 | 102 |
| $H_2O$ | 64 | 82,3 | 17,7 | 113 |

uranyl nitrate. On the other hand, it was markedly decreased in the 1 % solution of hydrogen peroxide.

There is no special need to enter into more details. Summarizing the facts, we conclude that dangerous cell poisons (KCN) and salts of heavy metals (uranyl nitrate) greatly affect the $X_1$ generation. Colchicine, although an active polyploidizer, did not enhance the X-ray influence.

*Mutation rates.* — The methods applied in this set of experiments prevent a maximum production of mutations. None the less, the mutation rates (Table 2) are generally increased by the various pre-treatments. The dry series gave 2,95 % chlorophyll mutations in the $X_2$ generation. This corresponds to a total mutation frequency of about twice that figure. Calculated per r-unit and spike progeny, the mutation rate is $3{,}9 \cdot 10^{-6}$, a value well in agreement with the rates obtained by STADLER in 1929 as well as by later authors (cf. GUSTAFSSON, 1947, p. 42).

The four highest rates are induced by the diluted solutions of KCN, the concentrated solution of $H_2O_2$ and the saturated solution of butter-yellow. They reach a figure of about three times that of the dry series (9 against 3 %, or $12 \cdot 10^{-6}$ against $4 \cdot 10^{-6}$ if calculated per r-unit and spike progeny). These rates may, as mentioned above, have been even

TABLE 2. *The combined effect of chemical pre-treatment and irradiation (7.500 r). Rates and types of mutation in the $X_2$ generation.*

| Pre-treatment | Number of plant progenies | Number of spike progenies | Number of mutants | Mutation rate per spike progeny % | Calcul. per r-unit · $10^{-6}$ | Type of mutation | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Albina | Viridis | Xantha | Albo-viridis | Tigrina | Striata | Others |
| Dry seed series ..... .... | 246 | 611 | 18 | 3,0 | 3,9 | 8 | 8 | 1 | 1 | — | — | — |
| Colchicine 0,1 % ..... | 222 | 587 | 30 | 5,1 | 6,8 | 13 | 7 | 5 | 1 | 2 | 2 | — |
| » 0,05 » ..... | 227 | 593 | 17 | 2,9 | 3,8 | 6 | 5 | 2 | 3 | — | — | 1 |
| » 0,01 » ..... | 193 | 530 | 20 | 3,8 | 5,0 | 7 | 5 | 2 | 2 | 2 | 1 | 1 |
| » 0,005 » ..... | 226 | 602 | 18 | 3,0 | 4,0 | 4 | 4 | 3 | 1 | 2 | 4 | — |
| Potassium cyanide 0,01 mol. | 87 | 236 | 6 | 2,5 | 3,4 | 3 | 2 | — | — | — | 1 | — |
| » » 0,001 » | 71 | 194 | 17 | 8,8 | 11,7 | 6 | 6 | 3 | — | — | 1 | 1 |
| » » 0,0001 » | 155 | 366 | 23 | 6,3 | 8,4 | 10 | 6 | 4 | 2 | 1 | — | — |
| Uranyl nitrate 0,001 mol. .. | 241 | 638 | 27(—29) | 4,2 | 5,6 | 16 | 7 | 2 | 2 | — | — | (2) |
| » » 0,0001 » .. | 100 | 285 | 16 | 5,6 | 7,5 | 8 | 3 | 2 | 3 | — | — | — |
| Hydrogen peroxide 1 % .. | 100 | 265 | 18 | 6,8 | 9,1 | 7 | 9 | 1 | 1 | — | — | — |
| » » 0,5 » .. | 143 | 371 | 13 | 3,5 | 4,7 | 6 | 6 | — | — | — | — | 1 |
| Butter-yellow ..... | 150 | 391 | 27 | 6,9 | 9,2 | 13 | 7 | 2 | 2 | 1 | 1 | 1 |
| $Fe_2(SO_4)_3$ ..... | 100 | 272 | 12 | 4,4 | 5,9 | 5 | 6 | — | — | — | 1 | — |
| pH = 3 ..... | 204 | 533 | 26 | 4,9 | 6,5 | 14 | 8 | 2 | — | 1 | — | 1 |
| pH = 10 ..... | 202 | 539 | 18 | 3,3 | 4,5 | 9 | 3 | 3 | 3 | — | — | — |
| $H_2O$ ..... | 187 | 498 | 19 | 3,8 | 5,1 | 11 | 4 | 1 | 1 | 1 | 1 | — |
| | 2854 | 7511 | 325(—327) | 4,3 | 5,8 | 146 | 96 | 33 | 22 | 10 | 12 | 6(—8) |

more increased by an appropriate co-ordination of pre-treatment and time of irradiation. The figure for the water treatment does not surpass the dry series by more than 30 %, against a value of 200 % or more obtained by STADLER and GUSTAFSSON in their experiments.

According to Tables 1 and 2, mutation rates and chromosome disturbances (manifesting themselves by a decrease in $X_1$ fertility) generally run parallel. There is however one remarkable exception. In spite of its pronounced $X_1$ effect the most concentrated solution of potassium cyanide (0,01 mol.) does not produce more than 2,5 % mutations against 8,8 and 6,3 % in the 10 and 100 times weaker solutions. The value lies even below that of the dry series. The difference between the two highest concentrations gives a P value of about 0,001. The strongest and the weakest solution still show a P value between 0,05 and 0,02.

This result is of profound interest, since it indicates that an increase of chromosome aberrations — translocations, inversions, deficiencies — does not necessarily raise the amount of detectable mutants. It argues against the view that visible mutations are nothing but chromosome re-arrangements or, at least, that they are always connected with the origin of chromosome disturbances. In this special case we conclude that concentrated solutions of potassium cyanide increase the rates of chromosome breakage, although the genic material does not react with an increase of visible mutants.

AUERBACH and ROBSON (1947), studying the effects of mustard-gas substances, were able to prove that lewisite causes a high mortality of the treated *Drosophila* flies and their progenies but does not augment the rate of recessive X-chromosome lethals. Future experiments will possibly reveal that such mutations arise after special optimum treatments but decrease in number when the dose is too high or too weak. Anyhow, the combination of X-rays and potassium cyanide in a medium concentration induces a maximum number of mutations.

The pre-treatment with a strong colchicine solution (0,1 %) highly increases mutability. This is raised from 3,0 to 5,1 % (from 3,8 to $6{,}8 \cdot 10^{-6}$, if calculated per r-unit). In the three lowest concentrations there is scarcely any effect at all. Their average mutation frequency amounts to 3,2 against 3,0 % (or 4,3 against $3{,}9 \cdot 10^{-6}$). As indicated by the next paragraph, there is however a pronounced influence on the types of mutation.

*Types of mutation.* — This experiment series was expressly designed in order to test certain conclusions advanced by GUSTAFSSON in

previous publications (1940, 1946, 1947, and others), viz. the postulated influence of cell environment on the mutations and mutation types appearing.

Instead of concentrating our studies on one or a few chemical compounds we thought it advisable to test the effects of numerous substances, salts of heavy metals, pronounced cell poisons, different seed conditions caused by a variation in the pH, and so on. In this way we hoped to be able to trace some regularities in behaviour and then in future experiments to extend the material of substances and cell conditions displaying special effects.

With regard firstly to the dry series, it may be stated that, as previously laid out, albina and viridis mutants form the majority of the chlorophyll mutants. In our material the alboviridis types were remarkably rare. This possibly depends on the large seed-size selected, or quite simply on the temperature conditions during the $X_2$ germination. In contrast to those described here, the experiments reported in GUSTAFSSON's paper of 1940 were carried out at a rather low room temperature (often below 10° C). It is now a well-established fact that low temperatures may cause a local inhibition of chlorophyll formation, so that in this way some viridis mutants are ranked as alboviridis. At high temperatures, on the contrary, some virido-albina mutants may be classified as viridis. In our experiments this temperature effect has no special significance, since the different $X_2$ series were sown exactly parallelly.

In other respects the dry series behaves rather normally. The xantha mutants occur ca. eight times as seldom as the albinas, and the very infrequent mutation types (alboxantha, tigrina, striata, maculata, etc.) did not arise at all.

Excluding the colchicine series for the moment, we find that the various pre-treatments have not markedly changed the mutation types. Against sixteen albina and viridis mutants and two rare mutants (89 and 11 % respectively) in the dry series, we find 175 albina and viridis mutants and 47 rare mutants (79 and 21 %) in the remaining series. This gives no significant difference ($0{,}5 > P > 0{,}3$). There is, however, a tendency in the weaker KCN series towards a widening of the mutation range. The case is similar in the uranyl nitrate series. A larger material obtained after even weaker treatments is required in order to settle this and other possible differences for certain.

In our view the colchicine series permit definite conclusions. The following survey gives the statistical values.

| | Albina + viridis | Rare and very rare mutants | Sum of mutants | Difference from the dry series |
|---|---|---|---|---|
| Dry series .. | 89 % | 11 % | 18 | — |
| $C_1$—$C_4$ .... | 60 | 40 | 85 | $P = 0{,}02$ |
| $C_2$—$C_4$ .... | 56 | 44 | 55 | $P = 0{,}01$ |
| $C_3$—$C_4$ .... | 53 | 47 | 38 | $0{,}01 > P\ (> 0{,}001)$ |
| $C_4$ ..... | 44 | 56 | 18 | $0{,}01 > P > 0{,}001$ |

There is, we consider, hardly any doubt that the colchicine series deviate considerably from the normal behaviour. It is also evident that the colchicine effect intensifies with increasing dilution. In this case it is not a matter of raised mutation rates, rather of a different course of the mutation process.

The conditions within the individual colchicine series are visualized below. In order to get exactly comparable figures we have referred them to 1,000 spike progenies.

| | Common mutants | | Rare mutants | | Very rare mutants | Sum of mutants per 1,000 spike progenies |
|---|---|---|---|---|---|---|
| | Albina | Viridis | Xantha | Albo-viridis | | |
| Dry series ..... | 13,1 | 13,1 | 1,6 | 1,6 | 0,0 | 29,5 |
| Colchicine 0,1 % | 22,1 | 12,0 | 8,5 | 1,7 | 6,8 | 51,1 |
| » 0,05 » | 10,1 | 8,4 | 3,4 | 5,1 | 1,7 | 28,7 |
| » 0,01 » | 13,2 | 9,4 | 3,8 | 3,8 | 7,5 | 37,7 |
| » 0,005 » | 6,6 | 6,6 | 5,0 | 1,7 | 10,0 | 29,9 |
| $C_1$—$C_4$ ..... | 13,0 | 9,0 | 5,1 | 3,0 | 6,5 | 36,8 |

Consequently, there is a distinct regularity underlying the figures. The increased mutation rate of the strongest concentration depends on a higher frequency of albinas, xanthas and very rare mutants. Viridis and alboviridis types are about as common as in the dry series.

With increasing dilution of the colchicine, the albinas and the viridis types distinctly diminish in frequency. The xanthas remain at a high level, similarly the very rare mutation types. The consequence is that the proportion of common, rare and very rare mutants is considerably altered. The weakest concentration of colchicine produces exactly half the number of albina and viridis characteristic of the dry series, against three times as many xanthas and — as a minimum approximation — about ten times the number of very rare mutant types.

Some of the colchicine mutants were entirely new in appearance. This was the case with (1) a *tigrina-like* mutant with the transversal

inactivation less conspicuous, (2) another *tigrina-like* variant possessing only two broad transversal stripes of yellowish-green colour, (3) a *special* mutation type beginning like a xantha but developing into a virido-albina type with green tip and white base, (4) an *alboxantha-like* type with the base distinctly yellowish-green, (5) another *alboxantha-like* variant with the yellow base short and the white tip very long.

But even within the viridis and xantha groups the phenotypical heterogeneity was striking, the colour varying from faintly white-green or yellow-green to an almost dark-green tinge, or from a light sulphur shade to a dark orange.

The viridis mutants can be divided into two groups, according to the »Côde des Couleurs» (KLINCKSIECK et VALETTE, Paris 1908), one comprising some of the colours 251—253 D (pp. 54—55), the other one comprising the colours 276—278 D (pp. 56—57). There is a certain difference in grouping:

| | Viridis A | Viridis B | No. of classified mutants |
|---|---|---|---|
| Dry series ..... | 80 % | 20 % | 5 |
| $C_1$—$C_4$ ..... | 25 | 75 | 16 |

This difference in behaviour argues for the conclusion that the viridis mutations are different in the dry and in the colchicine series.

Consequently, the colchicine treatment (1) widens the range of mutability, (2) increases the number of xantha types, (3) causes a gradual decrease (and change) in albina and viridis types.

Cytologically viewed, colchicine acts as a distinct cell poison. It interferes with and breaks down mitosis. In high concentrations its effect is so severe that the total X-ray mutability is essentially increased. In low concentrations it does not raise the mutation frequency, but it labilizes certain regions of the chromosomes (specific »genes») and stabilizes others. In this way it acts selectively on the mutability.

*Mutation type and sterility.* — The experiments analysed here were not specially designed to establish the connection between $X_1$ fertility and type of mutation (GUSTAFSSON, 1940, 1946 b). Owing to shortage of staff there was no possibility of determining more than part of the $X_1$ plants with regard to their fertility behaviour. In all, 103 albina, xantha and viridis mutants arose in their progenies. None out of eleven xanthas appeared in the offspring of normally fertile $X_1$ plants against four out of 55 albinas, in agreement with previous results.

The average fertility of the $X_1$ plants producing albinas, xanthas

and viridis types is given in the following table. The data do not show any significant differences, but they support the earlier conclusions.

| | Highly affected $X_1$ series | Slightly affected $X_1$ series | All series | No. of mutants |
|---|---|---|---|---|
| Average fertility of $X_1$ plants producing albina ..... | 73,0 % | 80,5 % | 75,3 % | 55 |
| Average fertility of $X_1$ plants producing xantha ..... | 67,9 | 80,0 | 72,3 | 11 |
| Average fertility of $X_1$ plants producing viridis ..... | 67,2 | 76,1 | 71,8 | 37 |

## DISCUSSION AND CONCLUSIONS.

In his papers of 1946 and 1947 GUSTAFSSON reviewed the data indicating the possibility of an experimental control of the mutation process. For barley they are as follows.

(1) The peculiar *alboxantha* mutation does not arise after an irradiation of dry seeds. It is not infrequent in germinating and hydrated seed series. Three different strains — Golden, Maja and »Ymer» barley (01513 $b_7$) — produced this mutation type in hydrated series. The significance of the difference is high ($P = 1{,}54 \cdot 10^{-4}$, according to a computation made by Professor J. B. S. HALDANE).

(2) The so-called *tigrina* type seems to accumulate in dry seed series. The difference »dry—wet» is not so pronounced ($P = 0{,}012$), but yet it indicates a certain restriction in origin. With this conclusion the material analysed here does not tally, since the wet series (distilled water) gave one tigrina mutant against none in the dry series. The tigrina type is rather common in the colchicine series (six independent cases). All the same, the characteristic two chlorophyll types of alboxantha and tigrina show a clear difference in mutual behaviour.

(3) The *xantha* mutants show a peculiar dependence on a distinct, although not very high, sterility in the $X_1$ generation. They accumulate in the offspring of plants having a fertility of 60—90 %. Normally fertile plants produce no or very few xanthas. The albina types, on the contrary, distribute at random over the whole fertility range. They frequently arise in the offspring of normally fertile $X_1$ plants. Using the data of series showing a slight irradiation effect (GUSTAFSSON, 1946 b, p. 302), we find that 122 $X_1$ plants producing albina possess an average fertility of $85{,}9 \pm 0{,}9$ %. Twenty-one $X_1$ plants producing xanthas show a fertility of $77{,}9 \pm 2{,}1$ %. The difference corresponds to a P value

below 0,001. These results were taken to indicate a connection between the type of genomatic change and the type of chlorophyll mutation.

(4) *Viridis* mutations were thought to be even more extreme with regard to their dependence on chromosome aberrations. This idea is supported by the fragmentary data of this paper.

(5) *Morphological* mutants divide into three groups: non-glaucous, erectoides and drastic mutants. The drastic mutation types, often improductive or sterile, appear in connection with a high $X_1$ sterility. The three groups are significantly separated from one another.

(6) As elaborated in this paper, a pre-treatment with colchicine, especially in low concentrations, distinctly widens the range of mutability, causes an increase in xantha mutants and induces other mutation types which do not normally arise in dry series. Some new mutants were discovered.

Scrutinizing all gathered data, we regard some of the points mentioned above as fairly conclusive. They indicate that for their origin the induced mutation types depend on the physiology of the irradiated seeds in a distinct way.

The important results published by KNAPP and KAPLAN (1942) and KAPLAN (1946; cf. also his publication of 1939) further strengthen this conclusion.

In their paper of 1942 the two authors examined the effect of irradiation on seeds of *Antirrhinum*, before and after an 8-hour's hydration (»Quellung»). Furthermore, they established the reversible character of the hydration. Mutants showing up in the seedling stage were more frequent, relatively seen, in series of dry seeds. Mutants detectable in later stages of development accumulated in series of hydrated seeds. There was, the authors claim, a distinct difference between the two types of mutation with regard to origin, a »groupwise mutability».

KAPLAN (1946) divided the mutants into two categories, an R-group (comprising chlorophyll aberrants and variants lacking in anthocyanin) and a Q-group (comprising growth and shape aberrants, as well as chlorophyll-free variants). He used pollen grains of *Antirrhinum*, irradiating them in the dry condition, after eight hours of hydration, after eight more hours of dehydration (a reversion to the dry condition) and, finally, after thirty-three hours of hydration.

Numerous R-mutants appeared after irradiation. Their number, however, in no way increased by the hydration. The Q-mutants, on the other hand, distinctly augmented in the series of 8 hour's hydration.

They were less frequent in the dry and dehydrated series. After 33 hours of hydration their number became low, too.

KAPLAN considered that the increase of Q-mutants after a short hydration and their decrease after dehydration result from reversible changes in the corresponding target regions (»Treffbereiche»). The low mutation rate after a long-time hydration is due, he thought, to an onset of developmental processes counteracting or covering the hydration effect and again lessening the sensitivity of the specific target regions.

The group mutability, as discovered in *Hordeum* and *Antirrhinum*, implies a peculiar phenomenon. It argues for a characteristic basic constitution of the numerous genes or chromosome parts responsible for individual types of mutation. According to KAPLAN, a number of genes possess some distinct fine-structure in common (for instance, dissociation constants). The change of environment leads to an »activation» of some of these genes.

Less peculiar is the metabolic dependence of the mutation type denoted as alboxantha. This mutant appears infrequently. Possibly the cell hydration required for its origin activates or re-organizes the fine-structure of one or a few special genes or chromosome regions within the genome. Similarly, the striking effects of low colchicine concentrations, described in this paper, may depend on an activation of a series of individual points distributed over the chromosomes, making them more easily mutate and give rise to rare types of mutation.

The ultimate object of the Svalöf experiments on induced mutations is to control and direct the production of vital mutations and by that means to change the agricultural plants at will. This may seem a rather pretentious task. But some results of the last ten years indicate that this object is not entirely out of reach. To take one example:

All erectoides mutants in barley are characterized by an increase in straw strength (a property of great agricultural value). A plant breeder wishing to apply a high nitrogen manuring or to combat severe lodging in an unfavourable type of climate has quite simply to start irradiation work on a large scale and then to pick out the re-appearing erectoides types. Since every tenth erectoides mutant attains or surpasses the yield of the mother strain, methodical experiments will rather rapidly establish a high-yielding erectoides type, automatically possessing the wanted superiority in straw strength. This does not amount to directing the mutation process, it is true, but it is a first approach to its definite control; it involves an intentional production of new valuable

properties by mutation. Moreover, we know the average sterility conditions facilitating the origin of erectoides mutants.

## SUMMARY.

The authors have analysed the combined influence of X-rays and various chemicals on the mutation process. They found that almost every chemical pre-treatment enhanced the mutation rate. An exception was a very strong concentration of potassium cyanide (0,01 mol.), which gave a lower mutation frequency than the dry control series, in spite of its marked effect in the $X_1$ generation. This indicates a medium concentration for the maximal production of mutations. Colchicine in low concentrations did not specially increase the mutation rates, but it distinctly widened the range of mutability. It changed the proportion of different mutation types, decreased the number of albina and viridis types, and augmented xanthas and very rare mutants. In addition, it induced some previously unknown mutations.

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# THE C-MITOTIC QUALITIES OF COLCHICEINE, TRIMETHYL COLCHICINIC ACID AND TWO PHENANTHRENE DERIVATIVES

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IN a couple of recent papers we have dealt with some problems connected with the c-mitosis. The nature of these problems often makes it necessary to approach them from the chemical as well as from the cytological side. In the first of these papers (STEINEGGER and LEVAN, 1947) the formula of colchicine was discussed and the c-mitotic testing of *iso*-colchicine was performed. By considering the thermodynamic activity of the threshold concentrations, i. e. the relation of concentration to solubility, it is possible, broadly speaking, to distinguish between specific chemical and unspecific physical activity. This opened up a new way for judging what groups of the colchicine molecule are responsible for its specific activity. (Concerning the formula of colchicine and other contributions in this direction the reader is referred to our paper mentioned above.) We were able to demonstrate that the ring C of the colchicine molecule is of decisive significance for the specific c-mitotic activity on *Allium* roots. If the keto- and methoxyl groups of ring C are interchanged, as is the case in *iso*-colchicine, the specific effect of colchicine disappears. The question is now how far the different substituents of the colchicine molecule influence its specific activity. Through the courtesy of Professor T. REICHSTEIN, Basle, who presented us with samples of colchiceine, trimethyl colchicinic acid and some synthetic model substances, it has been possible to follow this problem one step further.

The two colchicine derivatives, colchiceine and trimethyl colchicinic acid, represent two steps in the substitution by hydrogen of methyl and acetyl groups in the B- and C-rings of the colchicine molecule. If the common part of the molecule ($C_{19}H_{18}O_4$) is left out, the three substances are characterized by the following groups in the B- and C-rings:

| | | | |
|---|---|---|---|
| B-ring: | —$NHOCCH_3$ | —$NHOCCH_3$ | —$NH_2$ |
| C-ring: | —$OCH_3$ | —OH | —OH |
| | Colchicine | Colchiceine | Trimethyl colchicinic acid |

In addition, two synthetic phenanthrene derivatives (cf. MEYER and REICHSTEIN, 1944) were tested. The same method as earlier was used, the action of dilution series of the substances on growing root meristems of *Allium Cepa* being studied. Fixations were made after a treatment of 4 and 24 hours.

## I. EXPERIMENTS.

1. *Colchiceine.* — The solubility of colchiceine was determined as follows: 5 mg of colchiceine was gradually given so much water that all of it was dissolved when heated. After cooling off the solution kept clear. It was left several days, after which time the fluid was poured off from the crystals formed. The crystals were dried and weighed. Thus, the solubility of colchiceine in water at 20° C was determined as 0,6 ‰ or $1560 \times 10^{-6}$ mol/l.

The following concentration series was tested: 1560, 780, 312, 156 and $52 \times 10^{-6}$ mol/l (0,6—0,02 ‰). None of the solutions showed any strong or rapid toxic action. In the saturated solution the roots began to soften after 4 days and in the $^1/_2$ saturated after 6 days. No c-tumours were observed. The fixations after 4 hours showed no toxic effect, but after 24 hours the three highest concentrations acted slightly toxically.

No concentration gave complete c-mitosis, and only the saturated one showed any signs of c-mitotic tendencies. After 4 hours there often occurred in this solution a strong chromosome contraction and the mitotic poles were flattened. The same condition prevailed after 24 hours (Fig. 1 *a*), but here, too, all mitoses had bipolar spindles, so the anaphases were quite regular. It must be said that colchiceine is still weaker as a c-mitotic substance than *iso*-colchicine. The latter gave full c-mitosis down to $^1/_4$ saturated solution.

2. *Trimethyl colchicinic acid.* — This substance was in the form of monochlorhydrate, which was neutralized with 0,1 normal sodium hydroxide on dissolving. The pH was checked to ensure that the preparation did not deviate from neutral reaction. The solubility of the neutralized substance was determined as 1,4 ‰ or $4080 \times 10^{-6}$ mol calculated on free acid. The following concentration series was tested: 4200 (supersaturated), 2040, 1020, 410, 205, 100 and $40 \times 10^{-6}$ mol/l (1,44—0,014 ‰). This substance acted decidedly more toxically than the former one. After 4 hours the roots of the strongest concentration were already quite slack, and they began to soften down to $410 \times 10^{-6}$ mol/l.

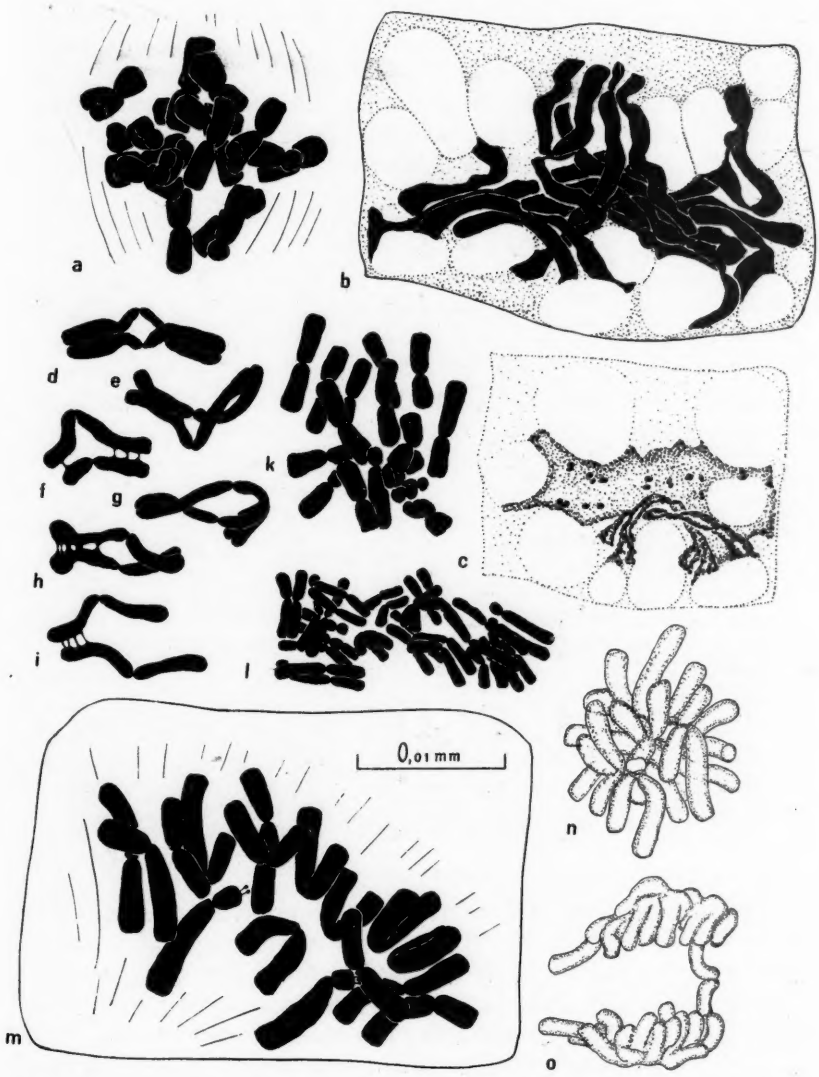


**Fig. 1. Chromosomes of *Allium* root tips treated in the following ways: *a*: colchiceine $1560 \times 10^{-6}$ mol/l for 24 hours, *b—l*: trimethyl colchicinic acid 4200 for 24 hours (*b*, *c*), 410 for 4 hours (*d—i*), $100 \times 10^{-6}$ mol/l for 24 hours (*k*, *l*), *m—o*: 3-oxymethylene-4-keto-tetrahydrophenanthrene $125 \times 10^{-6}$ mol/l for 4 hours (*m*) and 24 hours (*n—o*). — × 2250.**

After 24 hours they were soft in all concentrations from 4200 to $1020 \times 10^{-6}$ mol/l and were softening in 410 and $205 \times 10^{-6}$ mol/l. No c-tumours occurred.

The microscopic control showed strong poison effect in $4200 \times 10^{-6}$ mol/l in 4 hours. At the end of the same period 2040 and $1020 \times 10^{-6}$ mol/l showed vital structures in the meristem, while the external layers

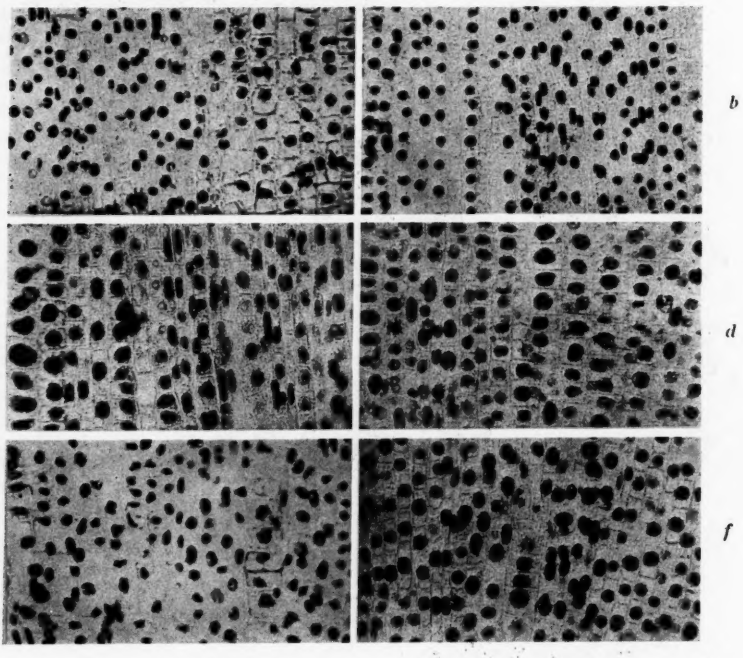


Fig. 2. Tissue samples of *Allium* root meristems. Treatments: *a—d*: trimethyl colchicinic acid 2040 (*a*), 410 (*b*), 205 (*c*), 40 (*d*) $\times 10^{-6}$ mol/l for 24 hours, *e—f*: 3-oxymethylene-4-keto-tetrahydrophenanthrene 125 (*e*), 62 (*f*) $\times 10^{-6}$ mol/l for 24 hours.

and older parts of the roots were destroyed. Lower concentrations gave no strong toxic effect in 4 hours. A treatment of 24 hours caused interesting changes of the roots: The two strongest concentrations, 4200 and $2040 \times 10^{-6}$ mol/l, showed extreme toxic changes: the nuclei were small, pycnotic and of irregular shape (Fig. 2 *a*); the chromosomes were liquid, usually they seemed not to have undergone any vital changes, metaphases and anaphases had stopped at the same stages as they were at the start of the treatment. The following two concentrations, 1020

and $410 \times 10^{-6}$ mol/l, had a characteristic influence, the nuclei were small, strongly stained, spherical in shape. All structures were decidedly pycnotic (Fig. 2 *b*). Chromosomes in contraction stages occurred and were often glued together to one pycnotic lump in the middle of the cell. The stickiness was striking. The next concentration, $205 \times 10^{-6}$ mol/l, deviated in type very much from the preceding. The resting nuclei were of normal size and contained delicate chromatic structures (Fig. 2 *c*). They were vital in appearance. No metaphases or anaphases were found but single prophases. Evidently the poison effect is weaker than in the former concentrations, the limit concentration having now been reached at which the cells may let the contraction stages despiralize into normal resting stages. The two weakest concentrations gave only little toxic effect in 24 hours. Some deficit in divisional stages and a certain general stickiness of the chromosomes were typical (Fig. 2 *d*). This series is characteristic of many types of chemical treatments of a living tissue. From both directions within the series the frequency of nuclear divisions decreases and reaches a minimum at $205 \times 10^{-6}$ mol/l. In the higher concentrations the vital processes have become so retarded that the divisional stages still linger although strongly changed; in the lower concentrations the toxicity is so weak that new mitoses may start.

The appearance of the chromosomes is stamped by the stickiness present all through the series. Figs. 1 *b* and *c* show a couple of typical metaphase pictures from the strongest concentration. The plasm is vacuolized, the chromosomes flow out towards the cell-wall. The liquefaction of the chromosomes is apparent from the way in which their outlines follow the curves of the vacuoles. In *b* the chromosomes are still strongly stained, in *c* their dissolution has proceeded further. At high focus there were visible in the latter picture two chromosomes whose centromere regions were strongly stained. The rest of the chromosomes formed one consistent nebulous mass in which no other structures were visible than the deeply stained centromeric regions. Figs. 1 *d*—*i* present a number of anaphase chromosomes from a weaker concentration, $410 \times 10^{-6}$ mol/l. They show a characteristic type known from treatments with different inorganic salts (e. g. litium nitrate; LEVAN, unpublished). The centromeric regions separate, but the chromosome arms stick together and have great difficulty in getting free from each other. In the lowest concentration the stickiness gives rise to frequent anaphase bridges.

Special attention was directed to the question whether c-mitosis

occurred in this substance. Throughout all the higher concentrations numerous tendencies to c-mitosis were seen, but evidently it is hard to decide whether the lumping together of the chromosomes often met with has anything to do with c-mitosis or whether it is dependent on quite another mechanism brought about by the liquefaction of the chromosomes. The toxic changes found after 24 hours are often of such an unspecific kind as not to admit of being immediately identified with c-mitosis. In such a low concentration as $205 \times 10^{-6}$ mol/l the chromosomes are found to be rather normal after 4 hours' treatment, their contraction is somewhat stronger than normally, but all anaphases are bipolar. As mentioned above, this concentration contains no mitoses after 24 hours. The next concentration, $100 \times 10^{-6}$ mol/l, has the same type after 4 hours, normal mitoses with somewhat excessively contracted chromosomes. After 24 hours, however, this concentration had, surprisingly enough, a high frequency of typical c-mitoses. The contraction was strong and the chromosomes were scattered without signs of any spindle (Fig. 1 *k*). A really convincing feature, however, is the occurrence of typical apolar c-anaphases (Fig. 1 *l*). Probably owing to the liquefaction of the chromosomes characteristic of this substance the size of the chromosomes was unusually small, part of the matrix material having probably been washed out of the chromosomes. Almost no normal spindle formation occurred in this treatment. The weakest concentration, $40 \times 10^{-6}$ mol/l, on the other hand, had quite normal mitoses after 4 and 24 hours.

Thus, it is evident that this substance exhibits c-mitotic activity far down in the dilution series. Although purely morphologically the c-mitoses are indistinguishable from c-mitoses induced by colchicine, the course of their induction is somewhat deviating, a point which may have a considerable theoretical interest. Colchicine gives c-mitosis in 15 minutes, completely and permanently, *iso*-colchicine and other more unspecifically acting substances give c-mitoses at the beginning of the treatment while later on in concentrations in the threshold region a certain resistance may be developed, resulting in normal mitoses in such concentrations as gave full c-mitoses at the beginning. In trimethyl colchicinic acid the condition is such that no c-mitoses were found after 4 hours' treatment; not until after 24 hours did a high frequency of c-mitosis occur. This situation makes the identification of the reaction somewhat difficult, since only one concentration above the threshold gave sufficiently typical mitoses after the lapse of 24 hours to permit a reliable decision as to the occurrence of c-mitosis.

3. *Two phenanthrene derivatives.* — The two substances studied were 3-oxymethylene-4-keto-tetrahydrophenanthrene and 3-methoxy-methylene-4-keto-tetrahydrophenanthrene, which are here abbreviated »oxy-compound» and »methoxy-compound» respectively. The oxy-compound is more soluble than phenanthrene, being dissolvable to $62 \times 10^{-6}$ mol/l; the methoxy-compound is of about the same solubility as phenanthrene, $17 \times 10^{-6}$ mol/l [according to OVERTON (1901), one part of phenanthrene is soluble in 300000 to 400000 parts of water, which corresponds to $14—19 \times 10^{-6}$ mol/l].

Of the less soluble substance, the methoxy-compound, a series was tested containing the concentrations 34, 17, 8, 4 and $1{,}7 \times 10^{-6}$ mol/l (i. e. $2—{}^{1}/_{10} \times$ saturated solution or $0{,}008—0{,}0004$ ‰). None of these concentrations acted poisonously. After 7 days, when the experiment was broken off, all roots were still turgescent and in growth. No c-tumours occurred. Nor did microscopic control after 4 and 24 hours' treatment show any poison effects; on the latter occasion the two highest concentrations held a small deficit of divisional stages, however.

C-mitoses did not occur, but the two strongest solutions, doubly saturated and saturated, showed tendencies to c-mitosis, more especially the chromosome contraction being greater than normally. In this respect this substance behaves as phenanthrene, which, according to ÖSTERGREN (1944), does not give c-mitosis but induces an increased chromosome contraction. In one treatment with saturated phenanthrene solution with excess of substance (corresponding to $28 \times 10^{-6}$ mol/l) the onion root showed first of all some growth retardation and later on normal growth; no c-tumours were induced. We did not test the roots on c-mitoses.

The oxy-compound was tested in the series 130, 62, 31, 15, 6 and $3 \times 10^{-6}$ mol/l (doubly saturated to ${}^{1}/_{20}$ saturated, $0{,}03—0{,}0007$ ‰). Here a distinct toxic action is found in the higher concentrations. A $130 \times 10^{-6}$ mol/l concentration already gives softening root-tips in 4 hours and quite soft roots in 24 hours; after 5 days, however, new fresh roots grow out in this concentration. Microscopically, the two strongest solutions already showed some toxic effect after 4 hours, and after 24 hours in a concentration of $125 \times 10^{-6}$ mol/l they acted strongly poisonously. The resting nuclei were small, dark-stained, pycnotic and irregularly shaped, and the divisional stages were liquefied (Fig. 2 *e*). The next concentration, $62 \times 10^{-6}$ mol/l, showed after 24 hours the very characteristic picture (Fig. 2 *f*) of normal resting nuclei and total absence of divisional stages. Between these two treatments, consequently,

lies the limit between, on one hand, the rapidly destructive effect which does not permit the divisional stages to despiralize and enter into resting stage but instead brings about a kind of »fixation» of the contraction stages, and, on the other hand, the weaker effect, which permits a normal return of the metaphases and anaphases into resting stage. In this substance a similar threshold to that in trimethyl colchicinic acid is represented by Figs. 2 *b* and *c*, i. e. 410 and 205 $\times 10^{-6}$ mol/l. In the case of the oxy-compound the two concentrations 130 and 62 $\times 10^{-6}$ mol are doubly saturated and saturated respectively; we here find a striking demonstration of a condition that often occurs in this type of experiments, viz. the significance of an undissolved excess of substance in a saturated solution. To such an excess the living cells respond differently and with a more extreme reaction than to a purely saturated solution. In the former case the toxic effect is so much greater that the normal structures of the cells are seriously damaged, in the latter case the cells succeed in keeping the normal vital processes working, metaphases and anaphases despiralize normally, the resting nuclei are not destroyed, etc. While the former changes are irreversible the latter will probably return to normal growth if the roots are put back into pure water.

In the two strongest solutions clear tendencies to c-mitosis occur after 4 hours. Contraction is strong and the spindles, although never absent, show irregularities (Fig. 1 *m*). The anaphases are always bipolar. The strongest solutions show after 24 hours such strong toxic changes that it is difficult to decide whether c-mitosis occurs. Figs. 1 *n* and *o* present one metaphase and one anaphase from this treatment. The chromosomes are transparent, weakly stained and sticky.

## II. DISCUSSION.

In the introduction we pointed out the possibility of distinguishing between c-mitotic substances acting through an unspecific physical effect and such as in addition have a specific chemical action. The determination of the thermodynamic activity of the threshold concentrations affords a means of distinguishing between these two types of mechanism (FERGUSON, 1939). The thermodynamic activity may be approximatively determined as the relation between threshold activity and solubility. As a limit value between physical and chemical action FERGUSON indicates a thermodynamic activity of 0,05, i. e. a threshold concentration lying 20 times inferior to the solubility.

Probably all substances have a certain amount of unspecific activity. This depends, above all, on the solubility of the substances. The thermodynamic activity of the thresholds of these unspecifically acting substances nearly always lies between 1 and 0,1, between saturated and 0,1 saturated solution. With decreasing solubility the value approaches 1. As the thermodynamic activity cannot increase above 1, a certain minimal solubility is a condition for the induction of the reaction.

Now, in these unspecifically acting molecules more or less specific groups may be introduced ($NH_2$-, OH-groups, etc.). As a result the substances may obtain an additive, chemical action, i. e. the substance will give effect in dilutions of the saturated solution which were earlier without effect, and the thermodynamic activity of the threshold concentration will get lower values. If it was situated at 0,1 before the substitution, it may have been moved to 0,01 ,if it was at 1, it may have become 0,1, and if the substance was ineffective owing to low solubility earlier, it may have become effective in saturated solution (thermodynamic activity = 1). The same chemically acting group need not give the same decrease of the threshold value in all combinations. It is clear that in large molecules one specific group is more »diluted« and as a consequence its influence is diminished. The behaviour of the aliphatic alcohols in toxicity and camphor reaction of yeast (LEVAN, 1947) is one instance of this condition. Through the introduction of the alcohol group the hydrocarbons become chemically effective. The chemical action is evident in methanol and ethanol. On increasing length of the aliphatic chain, however, the chemical action is overshadowed by the physical action of the aliphatic residue. Eventually even the physical activity disappears because of the low solubility of the higher alcohols.

Further, the chemically acting group of a compound may be masked. Aniline acts chemically, but through acetylation of the amino-group is deprived of its chemical action. Acetanilide therefore acts physically. The opposite situation may also be valid: through a certain change of a molecule an active group may be liberated and in that manner a chemical action may suddenly come to light. This case must be taken into consideration at the interpretation of the activity of trimethyl colchicinic acid, which compared with colchiceine represents a strong increase in c-mitotic activity. The colchiceine in saturated solution only gave tendencies to c-mitosis, i. e. a thermodynamic activity of 1, trimethyl colchicinic acid gave c-mitosis and toxic effect in $100 \times 10^{-6}$ mol/l, which with a solubility of $4080 \times 10^{-6}$ mol/l means a thermodynamic activity of 0,025.

As the activity of the colchicine was destroyed merely by an interchange of the groups in the C-ring, it is hardly surprising that the removal of one of these groups, as has happened with colchiceine, also means a disappearance of the activity. When in addition the N-acetyl group is removed, which leads to the origin of trimethyl colchicinic acid, the amino group of the B-ring is liberated. Since this group is masked in the colchicine, it is not correct to say that the »colchicine effect» re-appears; instead, it is a question of quite a new active group. This is also seen from the fact that the trimethyl colchicinic acid is characterized by a pronounced toxicity, which is totally absent in the colchicine at any rate when *Allium* is concerned. To what extent this toxicity is associated with the renewed c-mitotic activity cannot be decided. Toxicity and c-mitotic activity need not necessarily be combined. Colchicine, for instance, is c-mitotically active at 0,005 %, and even 20 % solutions are but little toxic to *Allium*. These properties, high water solubility, high c-mitotic activity and low toxicity, make colchicine unsurpassed as a polyploidogenic agent.

It will be seen that these results in *Allium* differ considerably from the results obtained with the same substances in animal material. Thus, BRUES and COHEN (1936), on liver cells, found that colchiceine showed $^1/_{30}$—$^1/_{40}$ of the activity of colchicine. Trimethyl colchicinic acid was, on the contrary, still inactive in 1000 times higher concentrations than the threshold value of colchicine. As is well-known also in the plant kingdom, great differences occur in susceptibility to colchicine. Bacteria, fungi and certain algae are resistant to colchicine. It is therefore not astonishing that animal cells may react differently from plant material. Especially striking is the pronounced toxicity of colchicine towards animal cells.

Another point is that the c-mitosis, as we use this term, is a determined, well-defined process, which can be followed in its details in meristematic cells of higher plants. Many of the published pictures of colchicine effect on animal tissue (especially in mammalia) show only rarely the arrangement of the chromosomes typical of c-mitosis. According to our opinion, it is not sure that all cases of »mitosis-stop», »mitosis-inhibition», »mitosis-poisoning», and so on, which have been reported in animal tissue *in situ* as well as *in vitro*, are identical with the reaction of c-mitosis. It may sometimes be the question of more general and unspecific toxic effects, also involving non-functioning of the spindle.

## SUMMARY.

Colchiceine gave only tendencies to c-mitosis in *Allium* roots. Trimethyl colchicinic acid induces c-mitosis in the concentration $100 \times 10^{-6}$ mol/l after 24 hours' treatment. Certain differences in the appearance of this c-mitosis from that of colchicine are ascribed to the fact that in the case of trimethyl colchicinic acid it is evidently another group of the molecule which is chemically active, viz. the amino group of the B-ring. Of the two tested phenanthrene derivatives only the more soluble one, 3-oxymethylene-4-keto-tetrahydrophenanthrene, had c-mitotic tendencies. The possibility of distinguishing between an unspecific physical and a specific chemical action in c-mitotic substances is discussed.

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# NOTES ON NUCLEOLAR CONDITIONS IN CEIBA PENTANDRA

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THE kapok-tree, *Ceiba pentandra*, has been studied cytologically by HEYN (1936), who counted 72 or 80 chromosomes in somatic cells, while the related species *Ceiba occidentale* had 88 chromosomes. Crosses between these two species have been performed by Dr. H. J. TOXOPEUS at the Agricultural Experiment Station at Buitenzorg, Java. These crosses succeeded easily and the $F_1$ progeny had a high pollen fertility, and a nearly normal seed setting (TOXOPEUS, pers. comm.). Since these results suggested a normal chromosome behaviour of the $F_1$ hybrid, it was thought to be of some interest to control the chromosome number of *Ceiba pentandra*.

Root-tip material from several specimens of different origin was fixed in LEVITSKY, NAVASHIN and CARNOY. LEVITSKY gave the best result. Cross sections were cut at 10 $\mu$. As starch, fats, plastids and other substances contained in the cytoplasm made the staining extremely difficult, different methods of staining were tried. NEWTON's gentian violet method and FEULGEN's reaction as well as a combination of both were tried but with unsatisfactory result. HEIDENHAIN's haematoxyline gave the best result for counting the chromosomes. Since the material showed rather interesting nucleolar conditions, longitudinal sections were cut at 15 $\mu$ and stained by the FEULGEN fast-green technique (SEMMENS and BHADURI, 1938) with a slight modification. A few drops of one-normal HCl in the fast-green solution gave a better differential staining.

The chromosome number of my material was constantly found to be 88, thus the same number as HEYN gave for *Ceiba occidentale*. Fig. 1, *a* represents a polar view of somatic metaphase. The chromosomes are rather small, the longest not reaching 2 $\mu$. Considerable size differences occur; the smallest chromosomes are about 0,7 $\mu$ in length. The chromosome thickness is about 0,3 $\mu$. Most chromosomes have median to submedian centromeric constrictions, but there also occur certainly some with subterminal constrictions. Several chromosomes

have satellite-like formations at one end, some of these differentiations being rather long.

At the study of the somatic chromosomes it was found that large nucleoli nearly always persisted through metaphase-anaphase. Although

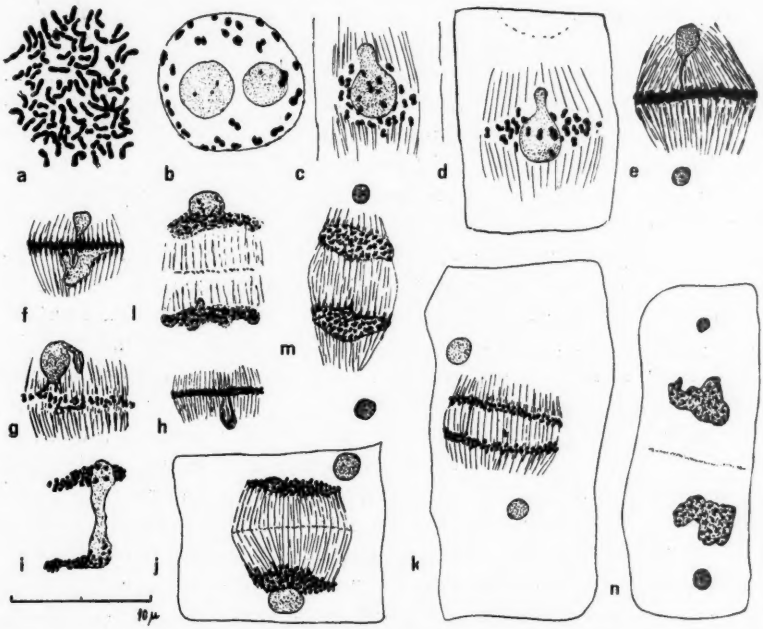


Fig. 1. *Ceiba pentandra*, mitosis of the root meristem. — *a*: somatic metaphase in polar view (2n = 88), the persisting nucleolus omitted; *b*: resting nucleus with 2 big nucleoli (note the small chromatic bodies inside the nucleoli); *c—d*: early metaphase; *e—h*: metaphase; *i—k*: anaphase; *l*: late anaphase; *m—n*: telophase; *c—d*: one large nucleolus starting to form a droplet; *e—h*: varying development of attached and free nucleoli; *e*: one typical stalk-nucleolus; *i*: nucleolar bridge between the anaphase groups; *j*, *l*: nucleoli attached to the anaphase groups, in *j* also one free nucleolus; *k*, *m*, *n*: one free nucleolus at each pole, in *k* one lagging chromosome and two chromosome bridges. — × 2100.

persisting nucleoli have been noticed in various plants by many authors (see TISCHLER, 1942, pp. 200—201), the present case seems to be rather extreme, and a closer study of the nucleolar behaviour was considered worth while. During the resting stage each nucleus usually contains one large nucleolus. Sometimes two large nucleoli may occur in the same nucleus (Fig. 1, *b*) or one large nucleolus and one or two small

nucleolar fragments, these latter often being in direct contact with some chromatic structure. At this stage no extra-nuclear nucleoli are present.

As seen from the figures, the chromosomes are tiny as compared with the nucleolus. Distinct chromosomes are present all through the resting stage, prochromosomic heterochromatin evidently occurring in all chromosomes. There is a »nucleolus court» (Nukleolenhof) present, which generally separates the large nucleolus from the chromosomes, but occasionally some of the largest chromosomes are seen to be in contact with the nucleolus. Almost regularly within the FEULGEN fast-green stained material 2 to 4 reddishly stained small bodies are observed in the nucleoli (Fig. 1, *b*). These formations probably represent the nucleolar organizers of some chromosomes, from which they may have been torn off at the fixing.

At the transition to metaphase when the nuclear membrane disappears, the nucleolus is usually not dissolved but persists. It is seen within the clusters of chromosomes during their congression to the equator (Fig. 1, *c*). Very soon the nucleolus changes shape on the spindle. From being spherical it becomes more oblongate or irregular in form. In the early metaphase it remains attached to some of the chromosomes forming the equatorial plate, and its behaviour is characteristic. If it is situated on one side of the plate, it grows out towards the nearest pole and finally forms one droplet on a narrow stalk, still attached to the equatorial plate. If it extends on both sides of the plate it will take dumb-bell shape, two drops being stretched out, one towards each pole. From these main types many variations may develop, two or more drops may be stretched out towards one or both poles. Some observed cases are recorded in Table 1. In addition to the attached nucleoli free ones are also found. They may occur from the beginning of metaphase, or they may take their origin as liberated drops from the attached ones. Table 1 shows that their frequency is higher at anaphase than at metaphase. Some attached nucleoli show very thin connecting stalks; they are evidently on the verge of loosening.

The variations in appearance of the nucleoli at metaphase may be seen from Fig. 1, *e*—*h*. Some very peculiar shapes are noticed, as lobated nucleoli with several attachments to the equatorial plate. One feature is common to all attached nucleoli: they seem to strive away from the equator towards the poles. The free nucleoli very seldom lie inside the spindle, they usually lie at the poles. At an early point they are evidently transported outside the spindle.

According to ÖSTERGREN's view (1945), the spindle in its property

of a tactoid structure has a tendency to remove from its interior anything which may disturb its equilibrium. This is according to ÖSTERGREN the reason for the characteristic spatial arrangement of the chromosomes within the equatorial plate, larger chromosomes lying closer to the exterior of the spindle, etc. These forces will mainly act in the transverse direction, as far as the chromosomes are concerned, because the chromosomes are suspended at the equator by the centromeres. In the case of nucleoli, however, these are, it is true, at first attached to the chromosomes at the equator, but the nucleoli, being of less viscosity than the chromosomes, will form drops which are transported outside the spindle. The division and transport to the poles of the persisting nucleoli, which have been difficult to account for by the various theories of mitosis (cf., for instance, SCHRADER, 1944, pp. 62—63), may thus be due to the same forces as effect the spatial distribution of the metaphase chromosomes on the equatorial plate, and have nothing to do with the forces of the anaphasic movement of the chromosome halves.

In most cells the nucleoli still persist after the division of the centromeres. They almost always lie on the outside of the anaphase

TABLE 1. *The occurrence of various combinations of attached and free nucleoli.*

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of attached nucleoli | | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 4 | Total of cases |
| Orientation | | — | — | — | — | — | — | — | 2+0 | 1+1 | 2+0 | 1+1 | 2+0 | 2+1 | 2+1 | 3+1 | |
| Number of free nucleoli | | 0 | 1 | 2 | 2 | 3 | 0 | 1 | 0 | 0 | 1 | 1 | 2 | 0 | 1 | 0 | |
| Orientation | | — | 2+0 | 1+1 | 2+1 | | — | — | — | — | — | — | 1+1 | — | — | — | |
| Number of cases | Metaphase | 5 | 8 | — | 3 | 3 | 6 | 4 | 2 | 10 | 3 | 2 | 2 | 2 | 1 | 2 | 53 |
| | Anaphase | 4 | 32 | 1 | 6 | — | 8 | 1 | 1 | 1 | — | — | — | — | — | — | 54 |
| Total | Metaphase | 5 | 14 | | | | 10 | | 19 | | | | | 5 | | | Total of types |
| | Anaphase | 4 | 39 | | | | 9 | | 2 | | | | | 0 | | | |

groups, whether they have as free drops already reached the polar region or are still in connection with the chromosome groups, forming a lump on its poleward side. It is quite characteristic that the attached nucleoli are now no longer stretched out from the chromosome groups (Fig. 1, *j*, *l*). There is evidently no longer any force pulling them away from the chromosomes. Instead they are often again drawn into the chromosome group. Sometimes, although rarely, there occur nucleolar bridges between the two anaphase groups (Fig. 1, *i*). Lagging chromosomes and chromosome bridges are found in some anaphases (Fig. 1, *k*).

At telophase the new nucleoli are developed. It often seems that the nucleolar substance develops all around the whole telophase chromosome group. The reddishly stained chromosomes seem to be embedded in a green mass (Fig. 1, *l*). However, later on this nucleolar matter concentrates to one large spherical nucleolus, usually present at the resting stage. At telophase the extra-nuclear nucleoli of the preceding nucleolar cycle may still be seen (Fig. 1, *m*, *n*). They are now decreasing in size, and will rapidly disappear.

In concluding this paper I wish to record my sincere gratitude to Dr. A. LEVAN for his valuable suggestions, criticism and help.

*Summary.* — (1) The somatic chromosome number of *Ceiba pentandra* is found to be 88, thus the same number as was given by HEYN (1936) for the related *Ceiba occidentale*. (2) The nucleolar cycle of *Ceiba pentandra* is described. (3) The transport of the persisting nucleoli to the poles during metaphase-anaphase is explained by the forces assumed by ÖSTERGREN (1945) to act within the spindle tactoid.

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# STUDIES ON THE SPERMATOGENESIS IN MUSCA DOMESTICA

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ALTHOUGH the knowledge of the cytology of Diptera is of great importance in connection with the genetic researches in *Drosophila*, only a few species outside this genus have been investigated. This has certainly been the result of the idea of Diptera being an unsuitable material for chromosome studies as compared with several other orders in Insecta, like Orthoptera and Heteroptera.

The oogenesis in Diptera has only been investigated in a few cases, as for instance in *Tipula* (BAUER, 1931), in *Drosophila* and *Calliphora* (GUYÉNOT and NAVILLE, 1933; NAVILLE, 1932), but has been shown to follow mainly the course characteristic of normal meiosis with typical chiasma formation.

On the other hand, the spermatogenesis of this order shows great variations, from cases with normal meiosis to those with very special conditions.

In the suborder Nematocera — the »lower» families — the meiosis is of the normal type both in the males and in the females (MOFFETT, 1936; PHILIP, 1942), except in the families Sciaridae and Cecidomyidae (WHITE, 1945) which show very special conditions. The meiosis of the latter can be derived from the normal type which appears in the other Nematocera, but on the other hand one cannot imagine it to be an extreme evolution from the meiosis type occurring in the »higher» Diptera (WHITE, 1945).

Probably exclusively or at least in most cases the suborder Brachycera — the »higher» Diptera — has the type of meiosis which DARLINGTON (1934) has described in *Drosophila pseudo-obscura* and which is characterized by the absence of pachytene pairing and chiasma formation. [Here I have used the classification of WHITE (1945) where he refers all Diptera, not belonging to Nematocera, to this suborder.] A similar behaviour is also described in certain Asilidae, Muscidae and Tachinidae (STEVENS, 1908; METZ and NONIDÉZ, 1921, 1923, 1924; KEUNEKE, 1924; EMMART, 1935; RIBBANDS, 1941).

Another characteristic of the order is the somatic pairing which

has been indicated by STEVENS (1908) and more exactly described by METZ (1916) who has shown this phenomenon in about eighty different Diptera, from the more primitive species as well as from the species that are more specialized. Thus, the origin of the somatic pairing is very likely earlier in the course of evolution than the differentiation to different types of meiosis.

STEVENS is the first one who has used *Musca domestica* in the cytological investigations of Diptera. She has shown that a pair of chromosomes sometimes remain unpaired, that these chromosomes are of different sizes and that they separate at the anaphase of the first meiosis, but divide themselves at the second meiosis. These heterochromosomes — in order not to mix the term heterochromosomes with the heterochromatic chromosomes, described below, I am going to use the word sex chromosomes in the following — are condensed during the growth stage of the spermatocytes, lying side by side without intimate pairing. The longer of the two chromosomes occurs in pairs in the oogonia and thus they are the *X* chromosomes. In STEVENS's examinations no early prophase stages are described. METZ (1916) has examined the somatic pairing in *Musca domestica*, and like STEVENS he has been able to distinguish a pair of sex chromosomes. KEUNEKE (1924) has described the shape of the chromosomes, and at the same time has shown that the sex chromosomes pair at the metaphase of the first meiosis.

All these investigations have treated special problems — as, for instance, the presence of sex chromosomes or somatic pairing — and they have given a general survey of a great number of Dipterous species. At present there are no investigations that more closely describe the course of spermatogenesis and of meiosis. As the statements of the authors, mentioned above, are opposed to each other and some parts of the meiosis are not yet investigated, it has seemed worth while to undertake another investigation of the spermatogenesis of this fly.

I wish to express my sincere gratitude to my teacher Professor GERT BONNIER for having given me this investigation to carry out and for valuable advice and criticism during my work.

*Material and methods.* — The material investigated was collected at Fiskebäckskil, Gävle, Örebro, Wiad and Öland during the summer of 1946.

The flies are bred in cages, size 20 × 20 × 20 cm, which consist of a wooden floor and a wooden frame, covered with insect net. The front side is made of a glass plate, which serves as a door. The adult

flies live upon sweet, white, moistened bread, that is put in a glass jar and placed in the cages. To avoid the bread becoming mouldy, a teaspoonful of nipagin solved in 96 % alcohol is poured over it. Every other day the bread is soaked with water, and about once a week new bread is placed in the cages. Twice a week some yeast suspension is dropped on the bread, as it is shown that flies which have not got yeast enough lay no eggs. When the flies have been about ten days in the cages, they begin to lay eggs. A 100 cc glass jar containing fresh horse manure, soaked with water and yeast suspension, is placed in the cage. Every day the jars with manure are changed in order to prevent too many eggs being laid in the same jar. The advantage of this method is that one can determine exactly how many days old the larvae and the adult flies are. Yeast suspension must be added every other day during the growth of the larvae, in order to keep them alive. It takes about three weeks from egg to adult fly, when bred in a thermostate of 25° C. These flies can be bred throughout the whole year, but in the months of November, December and January the development is much slower, and during that time one generation takes about a month. As even during this time the flies are placed in thermostates of 25° C, it cannot be the low temperature during the winter that prolongs the development. The cause is more likely to be the lack of light.

For the examinations of spermatogonia, testes from flies, newly hatched, have been used. In the larvae and the young pupae, the testes are very small, and I have not been able to localize them. The procedure of dissection is as follows: The fly is anaesthetized by ether, after which the abdomen is cut off and put into physiological saline solution (0,67 % sodium chloride) or Ringer solution for cold-blooded animals (DARLINGTON and LA COUR, 1942). It is very important for the big air sacs in the abdomen to be removed, so that the abdomen will be immediately penetrated by the solution; otherwise the testes will shrink.

A great many different fixatives have been used. For experiment material, which is to be stained in crystal violet, Randolph's CRAF (SMITH, 1943) has proved to be the most suitable, while for staining by the Feulgen method, fixation in modified Kahle (SMITH, 1943) gives the best results.

For making microscopical preparations the following methods have been used: (1) *The smear method* (DARLINGTON, 1942; SMITH, 1943). The testes are dissected in physiological saline solution and then they are transferred to a slide covered with a thin film of albumen. Here

they are spread over the slide with a flat-honed scalpel that is brought over the slide obliquely with the edge down. During the spreading it is important for the pressure to be concentrated to the sharp edge of the scalpel. After that the slide is rapidly transferred to a fixative, newly mixed in a petri dish. Too long a fixation is unfavourable, and often it is sufficient with a fixation of one hour. The slide can immediately be put into 70 % alcohol, after which staining takes place in the ordinary manner. (2) *The section cutting method.* Some testes have been fixed, embedded in paraffin after chloroform and have been sectioned with a thickness of 5—7 $\mu$.

The best staining method has proved to be crystal violet. Further a lot of Feulgen preparations have been made to find out nucleal reactions. In this case light green has been used as a counter-stain.

*The constitution of the testes.* — The testes of *Musca domestica* are two black or brown pear-formed organs, each one of which by the help of a vas deferens is united to an unpaired ductus deferens.

The testis has only one follicle (KÜKENTHAL, 1936) which in the adult fly is divided into different cysts, limited by connective tissues. In the apical part of the testis the imagines have a clearly marked spermatogonial region where new spermatogonial cells are continually being developed from an apical cell (KÜKENTHAL, 1936). After the spermatogonial region there comes a zone with spermatocytes, then along the wall of the testis come cysts where the cells are in the spermatid stage. The mature sperms are accumulated in the interior of the testis and in the lower parts of it. In the testes we usually have sperm packets, but in vasa deferentia the sperms are always free from each other. In favourable material all of these stages appear in the same section, and they are nearly all in serial order.

The testes of the pupae mainly contain only primary spermatogonia, i. e. spermatogonia that have not yet begun the cyst stage. Only a few cysts occur, but they may already have reached the stage of mature sperm.

In a cyst all or nearly all the cells are in the same stage of development. As all these cells in the cyst come from only one primary spermatogonial cell (KÜKENTHAL, 1936), which is the case in all Insecta, and as all the divisions pass away synchronically, the number of divisions can be determined by counting the cells in the cyst. The number of cells increases in a geometrical series: 1, $2^1$, $2^2$, $2^3$, $2^4$ ... $2^n$, where $n$ corresponds to the number of divisions. In most of the cases the number of spermatocytes has been about 64 and in a few cases

about 128. Thus there will be 5—6 secondary spermatogonial generations in *Musca domestica.*

Among the mature sperms and among the cysts are scattered a number of nutritive cells. These are of about the same size as the primary spermatogonia, and according to METZ (1932) they probably have the same origin.

*The chromosome set.* — The diploid chromosome number of *Musca domestica* has been determined by STEVENS (1908) to $2n = 12$, and later it has been confirmed by METZ (1916) and KEUNEKE (1924). The five autosome pairs are all metacentric — they have two long chromosome arms (WHITE, 1945). KEUNEKE has, in his material, determined the chromosome set to four metacentric and one acrocentric autosome pair. Having very different sizes and different long chromosome arms they can be distinguished without difficulty (Fig. 1). Classifications

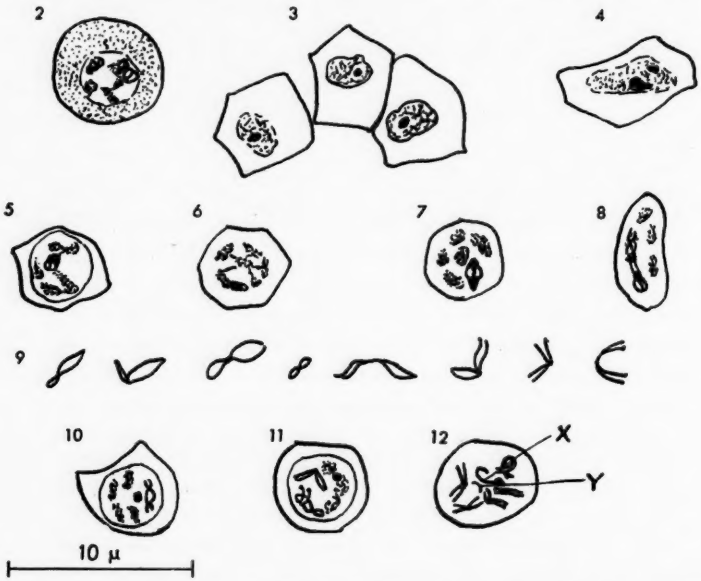
Fig. 1. Chromosome diagram of *Musca domestica.*

have been made partly from spermatogonial metaphases (Figs. 12 and 16), and partly from the metaphases of the first meiosis (Figs. 24, 26 and 28). Chromosome 2 shows a secondary constriction (Figs. 24 and 32). The sex chromosomes are usually strongly condensed, in the staining with crystal violet look like a big nucleolus (Figs. 24 and 25). Only in a few cases, where the *X* and *Y* chromosomes have been unpaired at the first meiotic metaphase, *X* has been seen to be a metacentric chromosome, and *Y* has been a very small dot-shaped or comma-formed one (Fig. 32). As it is very small, I cannot determine whether it is meta- or acrocentric.

*Spermatogonia.* — As mentioned above, the primary spermatogonia, which fill up most of the testes in the pupae, differ a great deal from the secondary spermatogonia. They have no cysts, and the cells are bigger and of a round shape. The chromatin is always deeply stained (Fig. 2). It seems as if the cells do not reach a resting stage with complete denucleination between the different cell divisions. Though the staining of chromosomes is stronger in the primary spermatogonia, the mitosis in other respects seems to follow the same laws as those of the secondary spermatogonia.

The secondary spermatogonia are smaller than the primary ones, probably owing to the repeated cell divisions. The cells have now got a five- or six-angled form (Figs. 3 and 4), not having had time to get round between the different cell divisions and having less space than in the primary spermatogonia.

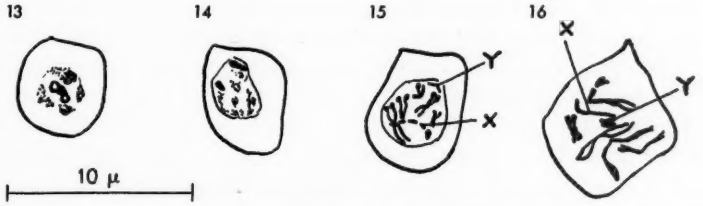
After the telophase the nucleus enters into a resting stage with dots




Figs. 2—12. *Spermatogonia.* — Fig. 2. A primary spermatogonial cell. A double nucleolus is seen and the chromatin is strongly stained. — Fig. 3. The resting stage. — Fig. 4. The resting stage. The two nucleolus-like structures are the sex chromosomes. — Figs. 5—8. The aggregate stage. One or two unstained nucleoli can be seen in the darkly stained sex chromosome mass. — Fig. 9. Separate autosome pair showing the somatic pairing. — Figs. 10—11. The aggregate stage. The cells show that the development of the separate chromosomes is not synchronous. — Fig. 12. The metaphase stage.

of chromatin equally distributed throughout the whole nucleus (Figs. 3 and 4). One or two nucleolus-like structures appear to be nucleal-positive in Feulgen staining and thus they contain chromatin. On the other hand, a true nucleolus with nucleal-negative reaction is not yet distinguishable. The chromatin material in the nuclei divides into a number of chromatin aggregates (Figs. 5, 6 and 7), which in every case eventually form six aggregates of which five are diffusely stained and one

is strongly stained in Feulgen (Figs. 6 and 7). This transformation can be followed successively, but in no cases more than five diffuse aggregates can be found. In some cases there are two deeply stained aggregates, but that is an exception. As mentioned above, the six chromatin aggregates correspond to the haploid chromosome number in *Musca domestica*. The strongly stained aggregate is made up of the nucleolus-like structure, which in this stage is usually composed of a nucleal-positive and a nucleal-negative part (Fig. 6); the latter is often double (Figs. 5 and 7). As is now known (Mc CLINTOCK, 1934; KAUFMANN, 1934), nucleoli usually appear at fixed loci in the chromosomes — in the secondary constrictions. Thus we must have a pair of heterochromatic chromosomes which are nucleolar organizers. The two




Figs. 13—16. *Secondary spermatogonia* from a fly from Wiad where the sex chromosomes are usually unpaired. See text.

nucleoli lying side by side, and not in different parts of the nucleus, are indirect evidence of the chromosomes being paired in this aggregate stage. The two true nucleoli increase, and sometimes they fuse already at this stage, becoming a big nucleolus which in some cases frees itself from the chromatin material. This only occurs exceptionally. Usually they are connected with the heterochromatic chromosomes during the whole stage. The five other chromatin aggregates are diffusely stained and have no nucleoli.

In the testes of a fly from Wiad, the heterochromatic chromosomes were unpaired in about 23 per cent of the spermatogonial cells (Fig. 14). In most cases I have found no nucleoli in these cells, but in one cell of this fly, where the two heterochromatic chromosomes have formed one mass, a nucleolus was seen at each side of the heterochromatic aggregate (Fig. 13). Even in this last case there has probably been no true pairing between the chromosomes. Also in latter stages — in spermatogonial metaphases — these testes have shown unpaired heterochromatic chromosomes (Fig. 15). As the unpaired heterochromatic chromo-

somes form two masses of different sizes, it is obvious that they correspond to the sex chromosomes, described by STEVENS (1908).

The chromatin aggregates become transformed to the long prophase chromosomes (Figs. 8, 10 and 11). At first, thread-like lines become visible in the round chromatin aggregate (Fig. 8), and gradually form a long rolled-up chromosome pair. In some places the two chromosomes of a pair separate from each other, but in almost all cases they are joined in the middle of the chromosome — this place perhaps corresponds to the primary constrictions. They are also joined at the ends of the chromosomes. All chromosome pairs are developed in different velocities and there are often cells where the big V-shaped chromosome is distinct, while all other chromosomes are still diffuse, and the pair of sex chromosomes a round mass (Figs. 10 and 11). In most cases it is the long chromosomes that appear first, but it sometimes occurs that one of the other chromosome pairs develops in advance (Fig. 11), thus showing that there is no synchronism in the development of the different chromosomes.

When the chromosomes go into the metaphase stage they have a loose pairing with each other (Figs. 12, 15 and 16). Only the primary constrictions and the ends of the chromosomes hold together. A somatic pairing, like that in *Drosophila*, where homologous chromosomes lie near each other without sections in intimate pairing, is never to be found at the spermatogonial metaphases in *Musca domestica* where the pairing is always intimate in some sections of the chromosomes even at the metaphases. Moreover, the chromosomes are often coiled round each other (Fig. 9). The sex chromosomes are sometimes paired (Fig. 16), but in some cases they are unpaired, often lying in different parts of the nucleus (Fig. 15). At the metaphase stage there are no nucleoli.

The anaphase is normal. At the telophase I have not been able to determine whether the homologous chromosomes are paired or not.

*Spermatocytes.* — The meiotic prophase does not run to a normal schedule owing to the somatic pairing. Early stages, as leptotene and zygotene, do not occur, the chromosomes being already paired before the beginning of meiosis. The nomenclature, used in normal meiosis, can hardly be employed on the meiotic prophase stages in Diptera. Also in the prophase stages, as diplotene and diakinesis, there are no exact parallels, owing to the absence of chiasma formation in the males of the higher Diptera. RIBBANDS (1941) has described six different stages in meiotic prophases in *Habropogon appendiculatus*, and they

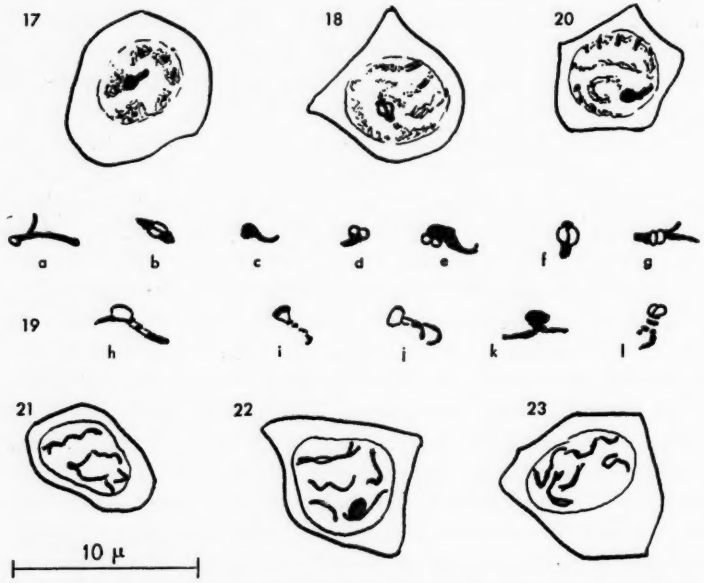
are referred to as stages A—F. I am going to follow his classification and not until later am I going to discuss their probable homology to the stages found at normal meiosis.

The different stages are described as follows: *Stage A* is a direct continuation of the last spermatogonial telophase. No cell growth has occurred. The nucleus contains a number of fine threads, which anastomose in various directions. — *Stage B.* The chromosomes separate into aggregates, the number of which corresponds to the number of haploid chromosomes. There is a nucleolus. The chromosomes are connected with each other and also with the nucleolus by fine threads, which may either be artefacts or true connections between non-homologous chromosomes. According to RIBBANDS, they are probably examples of non-homologous association. The sex chromosomes are attached to the nucleolus. The cells increase rapidly in volume. — *Stage C* has the longest duration of all the prophase stages. The increase of cell volume is very rapid. The autosomes become elongated to long threads with chromomeres of various sizes. The number of visible threads corresponds to the haploid number of autosomes (five). The nucleolus is large and connected with the sex chromosomes. There is non-homologous association between the chromosomes, as the sex chromosomes, centromeres and chromosome ends are attracted to each other. — *Stage D.* Stage C passes gradually into stage D, the chromosomes being shorter and thicker. No split is yet visible between the closely paired autosome threads. — *Stage E.* At this stage the nucleolus disappears. The sex chromosomes are still more chromatophile than the autosomes. Short parts of the latter can be seen to be double. The non-homologous association between the chromosomes is reduced. Stage D and E each occupy about a quarter of the whole prophase. — *Stage F* is of very short duration. The autosomes are contracted and become smoother in contour. The sex chromosomes form a sphere. There is no visible sign of any split in the stained bodies, each of which presumably represents a pair of autosomes. The non-homologous associations are greatly reduced in number. From this stage the cell passes quickly into the first metaphase.

Also in *Musca domestica* the meiotic prophase follows mainly this schedule. Differences are to be found in some details only. Stage A entirely resembles a secondary spermatogonial prophase, and only by counting the cell generation in the cysts can these stages be found. The chromosomes are fine threads and it is difficult to find out in the preparations if there are some anastomoses.

As in the spermatogonia there is a typical aggregate stage (Fig. 17) which corresponds to stage B of RIBBANDS. The different aggregates are connected with fine threads (cf. Fig. 6 in the spermatogonia) which at a later stage disappear, and the aggregates in the cells become free from each other. The sex chromosomes are heterochromatic and have a double nucleolus (Fig. 19).

Soon the aggregates are dissolved and unrolled to long chromo-




Figs. 17—23. *Primary spermatocytes at prophase stages.* — Fig. 17. Stage B. — Fig. 18. Stage C. — Fig. 19. The sex chromosome pairs from different cells showing negative heteropycnosis in *h*, *i* and *l*. Unpaired sections and double nucleoli are also to be seen. — Fig. 20. Stage C. — Fig. 21. Stage D. — Fig. 22. Stage E. — Fig. 23. Stage F.

some threads with distinct chromomeres (Fig. 20). In an early thread stage the chromosomes are very faintly stained with the exception of a few loci which undoubtedly are heterochromatic (Fig. 18), and which probably correspond to the primary constrictions. They number as many as the autosome pairs and are always double, so it is easy to see that the chromosomes are paired in these places (Fig. 18). The nucleoli are very distinct and have the same appearance as in the spermatogonia. At this stage — which corresponds to RIBBANDS's stage C — there is

sometimes a fusion of the nucleoli, but they are still connected with the sex chromosomes, which are now separated into a paired and an unpaired part (Fig. 19). There is no attraction between non-homologous chromosomes. The cells have reached maximal sizes and the nuclei are large and round and filled with liquor. It seems as if the chromosomes have been pressed out to the nuclear membrane. As in *Habropogon* this stage is the longest during the prophase.

Then the contraction of the chromosomes takes place very rapidly and in stage D the threads are separated from each other. As the number of threads corresponds to the haploid number of autosomes (Fig. 21), there are bivalents, and each thread consists of a pair of autosomes.

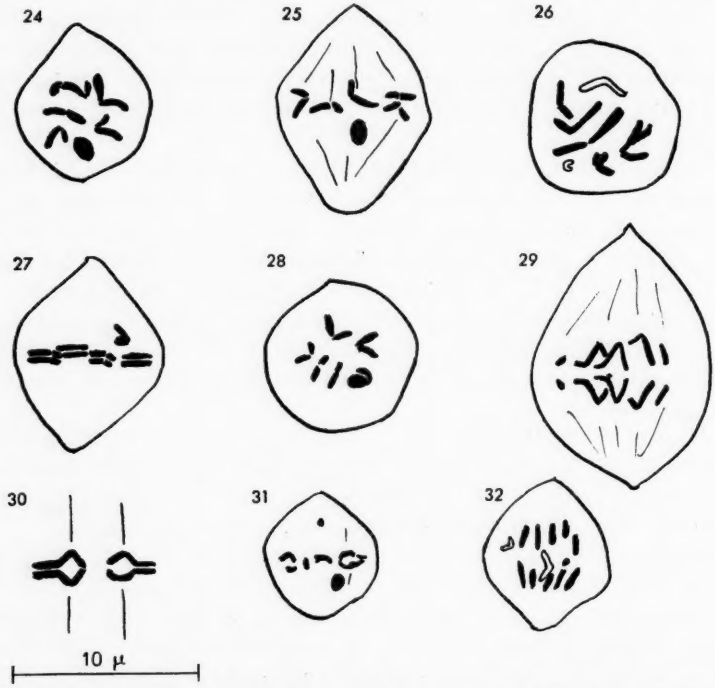
At a later stage sections of the chromosomes in the bivalents begin to separate and form a kind of loop formation. In other parts of the chromosomes the pairing is still very intimate. Now the nucleoli are free from the sex chromosomes, which are still positively heteropycnotic (Fig. 22). The distal parts are unpaired, but the proximal parts paired (Figs. 19 *a*, *g*, *j* and *l*). As in *Drosophila melanogaster* (DARLINGTON, 1937) and in many other species, *X* and *Y* are composed of a proximal, homologous part, and the distal parts are probably differentiated.

During the contraction process of the chromosomes the loop formation from stage E disappears, and the chromosomes of a bivalent will once again lie so closely paired that no split is to be seen in the bivalents (Fig. 23). It is very difficult to decide if this depends on a new tendency in the pairing of the chromosomes, or if the continued contraction makes it impossible to distinguish the split between the chromosomes. The nucleolus has disappeared, and the cell rapidly enters a metaphase stage.

The only true differences between the meiotic prophase in *Musca domestica* and in *Habropogon appendiculatus* is the absence of non-homologous association between the chromosomes in *Musca*, a phenomenon which, according to RIBBANDS, is strongly pronounced in *Habropogon*. The threads between the chromosomes in the aggregate stage may possibly be of the same nature, as may also the situation of the chromosomes in Fig. 21 be interpreted by association between chromosome ends. The latter figure does not express the usual behaviour in this stage. RIBBANDS has not described any double nucleolus in *Habropogon*.

In contradistinction to *Drosophila pseudo-obscura* (DARLINGTON,

1934) a clear metaphase plate is formed at the first meiosis in *Musca domestica* (Figs. 24, 26 and 27). That is not necessarily a species difference between *Musca* and *Drosophila*. The reason why *Musca* can show many stages that are not found in other Diptera, depends perhaps on the fact that investigations are made in November and December, when development takes place much more slowly than during the sum-



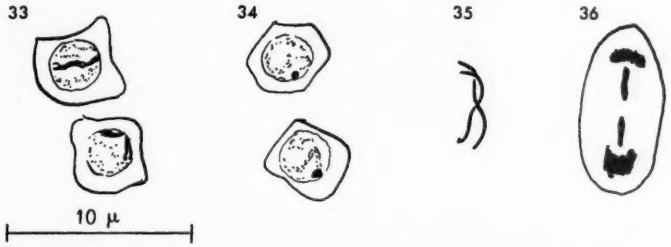

Figs. 24—32. *Primary spermatocytes at metaphase and anaphase stages.* In Fig. 26 the sex chromosomes are unpaired and only drawn in contour. Fig. 30 shows the pairing of two chromosomes. In Fig. 32 the sex chromosomes begin to lag.

mer. Then every stage is of longer duration and stages that never appear in summer — as, for instance, the first meiotic metaphase — are to be seen in these preparations. This shows that it is of great importance to call forth experimentally a slow development in animals which are to be investigated cytologically.

At the metaphase stage there are no nucleoli. The sex chromosomes are usually paired (Figs. 24, 25 and 27). They often have a round

shape (Figs. 24 and 25). In this case no split between the two chromosomes is to be seen. Sometimes the *X* chromosome is relatively long and at one end there is a small lump, which consists of the *Y* chromosome (Fig. 27). While all the autosomes are in the same developmental stage during the metaphase, *X* and *Y* can be before as well as after in their development (Figs. 25 and 27). This behaviour can be seen much better at the anaphase, the sex chromosomes sometimes lying closer to the poles than the autosomes (Fig. 31), sometimes — and this is the most usual case — lagging in the equatorial plate when the autosomes have already reached the poles (Fig. 32). As I have never found non-disjunction, *X* and *Y* must separate before the cell has divided.

At the metaphase the primary constrictions in the chromosomes are very distinct, and in the second chromosome a secondary constriction




Figs. 33—36. *Secondary spermatocytes.* — Fig. 33 with heterochromatic *X* chromosomes and Fig. 34 with heterochromatic *Y* chromosomes. — Fig. 35. A single chromosome with the typical pairing of the second meiosis. — Fig. 36. Anaphase with the two *X* chromosomes lagging.

can also be seen, as mentioned above. The latter constriction has no nucleolar organizer. This chromosome resembles, in this condition, the chromosome II in *Drosophila melanogaster*, described by KAUFMANN in 1934. The secondary constrictions which ought to occur in the *X* and *Y* chromosomes cannot be distinguished, as the strong contraction makes it impossible to discern details. If the chromosomes are rolled up from the round formation, they are very often heteropycnotic (Figs. 19 *h*, *i* and *l*), so in this case it is also impossible to settle if they have secondary constrictions, with nucleolar organizers. At late metaphase the autosomic bivalents begin to show a clear split (Figs. 26 and 30), along some parts of the chromosomes. This time there is no loop formation of the kind that occurs in stage E.

This split is the beginning to the anaphase, and the centromeres now separate, going to different poles (Figs. 29, 30 and 32). Then we

get a chromosome figure that partly corresponds to the figures described by DARLINGTON in *Drosophila pseudo-obscura.* In *Musca,* however, no division into chromatids can be seen.

The telophase then passes normally. None of the separate chromosomes can be seen. The interkinesis stage between the first and the second meiosis is of very short duration, the cells very soon going into the prophase of the second meiosis. In this stage, as in the interkinesis stage, the sex chromosomes are positively heteropycnotic, and it is now easy to see that in one half of the cells there are long heterochromatic chromosomes — the *X* chromosome — and in the other half there are short ones — the *Y* chromosome (Figs. 33 and 34). Thus *X* and *Y* have separated during the first meiosis, a process which was shown by

Figs. 37—40. *The spermatids and the transformation to mature sperms.* — The sex chromosomes form a separate nucleus which in Fig. 39 has fused with the big nucleus. — Fig. 40. The chromatin shows a crescent-shaped formation which gradually stretches out and forms the greater part of the mature sperm.

KEUNEKE (1924) who followed the chromosomes during the anaphase of the first meiosis. During the second meiotic division the autosomes behave normally, and chromosome figures, typical of second meiotic anaphase, are often seen. Here the metacentric chromosomes have the figure of a cross (WHITE, 1945), owing to the sister chromatids repelling each other (Fig. 35). At the anaphase of the second meiosis the sex chromosomes are always later in development than the autosomes (Fig. 36), probably owing to their being heterochromatic. The consequence is that at late anaphase, when the autosomes have begun their telophase, the sex chromosomes lag and form a special chromatin mass, separated from the other chromosomes. The nuclear membrane is formed round both their structures.

*Spermatids.* — When the conversion into sperms begins, the sex chromosome as well as the mass of autosomes lie along the nuclear membrane at one side of the cell, forming a crescent-shaped formation

which gradually stretches out and makes up the greater part of the complete sperm (Figs. 39 and 40). This special condition will be described in a later paper.

*The chromosomes in somatic tissues.* — I have not undertaken a particular investigation of the behaviour of chromosomes in different somatic tissues, but in a section that I have got of a ductus deferens there can be seen that in all the cells two of the chromosomes in the resting stage were heterochromatic. These chromosomes had different sizes; one of them was V-shaped and the other rod-shaped (Fig. 41). They are probably the sex chromosomes, which thus also in somatic tissues are heterochromatic. The chromosomes are not paired, but they often lie near each other, and only in a few cases are they seen in different parts of the cell. There must be further investigations in

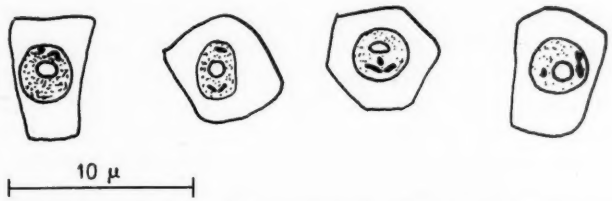



Fig. 41. *Some cells from ductus deferens* with heterochromatic sex chromosomes.

order to establish the extent to which the sex chromosomes have somatic pairing in the resting stage.

*The nucleolus.* — The descriptions of the behaviour of the nucleolus during the spermatogonial and spermatocyte stages in Diptera are very contradictory. EMMART (1935) has shown in *Anastrepha ludens* that the nucleolus is made up of two lobes, but at the same time she says that these two masses of chromatin seem to be the first chromosome pair. As the preparations are stained in haematoxyline, she has not been able to decide if there is a differentiation in plasmosomic and basophilic material.

On the other hand, METZ and NONIDÉZ (1921, 1923) have shown this differentiation in *Asilus sericeus* and *A. notatus.* In a paper of 1932 METZ has described the nucleolus as a dense mass which later differentiates into two parts, one of which is stained darker than the other. The darker part and the chromosome threads, which are attached to the nucleolus, constitute the sex chromosomes. The lighter part is true nucleolar material.

By Feulgen staining there can be shown in *Musca domestica*, firstly that one pair of the chromosomes is heterochromatic, secondly that this pair carries a nucleolar organizer (Fig. 19). This corresponds to the conditions mentioned above, described by METZ and NONIDÉZ in *Asilus sericeus*. On the other hand, in *Anastrepha* it cannot be decided whether the darkly stained bodies are heterochromatic chromosomes, or if that »nucleolar» figure is of a composed nature.

In Diptera it appears to be a common condition that it is the sex chromosomes which have a nucleolar organizer, as in the species of *Asilus*, *Lasiopogon bivittatus* (METZ and NONIDÉZ, 1924), certain *Drosophila* species (KAUFMANN, 1934) and *Habropogon appendiculatus* (RIBBANDS, 1941) among others. In *Lasiopogon* the lightly stained part of the nucleolus can be seen to be double in Feulgen staining. This confirms the condition, found in *Musca domestica*, that two nucleoli are formed, one from each of the two nucleolar organizers in the chromosome pair (Figs. 19 *b*, *d* and *e*). The nucleoli will soon fuse (Figs. 19 *h* and *j*) as by the somatic pairing they will lie close by each other.

RIBBANDS (1941) indicates that the intimate union between the nucleolus and the sex chromosomes depends perhaps on the fact that nucleolar material is generally attracted by heterochromatin. But he thinks himself that it may depend on the sex chromosomes having nucleolar organizers, which would not be necessary if the attraction hypothesis is to be accepted. That the nucleolus in *Musca domestica* is originally double, and that the two small nucleoli always arise close to each other, favours the belief that there is a specific nucleolar organizer in *Musca domestica*. Otherwise, one should be able to find small nucleoli somewhere in the heterochromatic parts of the chromosomes. The attraction between the nucleolar material and the heterochromatin, which is also described by MC CLINTOCK (1934) in *Zea mays*, can probably be seen at a later stage. The union between nucleolus—chromosome is more intimate than that usually occurring between the nucleolar material and the nucleolar organizer.

Often no heterochromatic parts of the sex chromosomes are to be seen, these parts of the chromosomes being entirely embedded in nucleolar material during the mitosis — as well as the meiotic prophase. A dark spot which perhaps corresponds to the heterochromatin from the sex chromosomes can sometimes be seen within the nucleolus (Fig. 19 *i*).

A similar figure is also described by METZ in *Asilus sericeus* and by RIBBANDS in *Habropogon*.

*The sex chromosomes.* — In the first spermatogonia already both the *X* and the *Y* chromosome are heterochromatic. With the exception of the condition, mentioned above, of the sex chromosomes in the cells of ductus deferens, I have made no investigations about the sex chromosomes in somatic tissues. There is a very distinct difference between the shape of the sex chromosomes in the ductus deferens and in the spermatogonia, as in the former case they are relatively long but in the latter more dot-shaped (Fig. 41). According to STEVENS (1908), the longer of the two chromosomes is the *X* chromosome. That *Y* is the smaller of the sex chromosomes is the most common condition in Nature, as this chromosome is often inert and then easily passes through structural changes, like deletions of short regions. Thus, the *Y* chromosome in *Drosophila melanogaster* is an exception from this rule.

In species with differentiated sex mechanism the *X* as well as the *Y* chromosomes are often heterochromatic. The best known example of this phenomenon is *Drosophila melanogaster*, in which the whole *Y* chromosome and $^2/_5$ of the *X* chromosome are heterochromatic (WHITE, 1945). We have probably a similar condition in *Musca domestica*, perhaps with the difference that the *X* chromosome is heterochromatic to a much greater extent. As heterochromatic parts in the chromosomes usually lack genes, the consequence is likely to be that we shall have only a few or perhaps no sex-linked genes in *Musca domestica*. Unfortunately no genetic analysis has been made as there are no sex-linked mutations known. But a similar case is described in a rodent where the *X* chromosome is totally heterochromatic and no sex-linked genes have been discovered (WHITE, 1945).

Owing to the sex chromosomes being heterochromatic also during the resting stage, it is easy to follow the course of their pairing. At the resting stage of the spermatogonia they are often unpaired, and the pairing begins at about the same time as the autosomes enter the aggregate stage.

For this pairing there are two possibilities: (1) the sex chromosomes lying near one another according to the common tendency of the heterochromatin to enter a non-homologous pairing; (2) that an intimate pairing of homologous sections takes place.

The second possibility must be the right one, as the nucleolar organizers in the testes of pure breeds always lie close to each other and there can be seen at late prophases a very closely paired section of the chromosomes where the chromomeres correspond to each other and an unpaired part of the chromosomes.

In the spermatogonia the pairing of the sex chromosomes is usually finished earlier than in the autosomes. According to this, the sex chromosomes generally are not close to each other during the metaphase of mitosis, while all the autosomes show an obvious somatic pairing. The sex chromosomes divide normally and pass to the poles at the same time as the autosomes.

In the fly from Wiad, mentioned above, the absence of the *XY*-pairing at the metaphase plate cannot be interpreted in the usual manner by the sex chromosomes being earlier in the development, as in this case the sex chromosomes already at the prophase show anomalous pairing conditions. Probably these chromosomes have not been paired.

At the prophase of the spermatocytes the pairing of the sex chromosomes runs exactly as in the spermatogonia and not until later do any differences occur. The sex chromosomes now are not in advance of the others, and they often remain paired when the autosomes have loosened from their pairing. As described in the paragraph of the nucleolus, it seems as if the sex chromosomes are usually embedded in nucleolar material which can surely lengthen the pairing stage. As the nucleoli in the spermatocytes are much bigger than in the spermatogonia and as they remain until the late prophase, this seems to be a plausible assumption. This hypothesis can also explain the great variation in the time at which the pairing of the *X* and the *Y* chromosomes comes to an end. In the spermatocytes it sometimes happens that the segregation of *X* and *Y* precedes that of the autosomes, but in most cases it does not begin until the autosomes have reached the poles. This lack in coordination between sex chromosomes and autosomes in the cells, which in other respects work at the same rhythm, can probably be explained by the nucleolar material in different cells having enclosed the sex chromosomes to different extents so that the development of the sex chromosomes will be earlier or later than that of the autosomes, as they meet with a smaller or a bigger obstacle. A similar discordance between the sex chromosomes and the autosomes has been described in some *Drosophila* species by METZ (1926) and DARLINGTON (1934).

In *Drosophila melanogaster* the pairing segment of *X* and *Y* is very small and the greater part of the chromosomes is differentiated, lying unpaired. As the sex chromosomes in *Musca domestica* are much contracted, it is difficult to determine the length of the pairing segment from meiotic figures. As the unpaired part of the sex chromosomes does not carry any primary constriction, it can be deduced that it is

the proximal part which is paired. This corresponds to the condition in *Drosophila melanogaster*. It cannot be determined cytologically whether there are any reciprocal chiasmata in the paired part, which DARLINGTON has described in *Drosophila*.

At the prophase of the second meiosis the sex chromosomes are once again heterochromatic (Figs. 33 and 34), but they are not so much contracted as at the prophases of the spermatogonia and of the first spermatocytes. The *X* chromosome appears as a long dark line across the whole nucleus (Fig. 33), and the *Y* chromosome as a short rod (Fig. 34). At the division stage the sex chromosomes always lag, a behaviour which seems to result in these chromosomes not being included in the nucleus (Figs. 37 and 38).

*The pairing of the autosomes.* — Originally METZ (1916) supposed that the somatic pairing occurred to a full extent in all Diptera. This interpretation has now been revised, as the somatic pairing is absent in, for instance, *Sciara* (METZ, MOSES and HOPPE, 1926) in the spermatogonia and the first spermatocytes, and in *Olfersia* (COOPER, 1944) between the sex chromosomes at the spermatogonial prophases. However, *Musca domestica* shows a very intimate somatic pairing in the spermatogonia as well as in the spermatocytes.

METZ and NONIDÉZ (1921) have been able to show that the somatic pairing is relatively intimate at the anaphase. At the telophase the pairing seems to be loosened again. After that the question is open at which time after the telophase the pairing begins again. BAUER (1932) has presumed that the pairing in *Tipula paludosa* begins in connection with the formation of aggregates. That is, however, an assumption, supported only by the fact that the number of aggregates corresponds to the haploid chromosome number. In *Musca domestica* it can be said without doubt that the chromosomes are paired during the aggregate stage, as the heterochromatic parts of the chromosomes, which can sometimes be seen at this stage (Fig. 18), always lie in pairs. Thus the pairing must take place either in the resting stage or in the passing from the resting stage to the aggregate stage. The second alternative is the most likely, as it seems as if the sex chromosomes pair during this change from resting stage to aggregate stage. But the behaviour of the sex chromosomes must not be taken absolutely as an example of that of the autosomes, as the sex chromosomes and the autosomes show no time co-ordination at the pro- and metaphases. But as it is clearly seen cytologically that the chromosome material is really moving in the nucleus during the change, the second alternative is more likely. Further-

more, aggregate stages seem to occur in all Diptera which are investigated cytologically.

When the single autosomes differentiate from the aggregates, they have a very characteristic form of pairing (Fig. 9). They are paired at the two ends, and at a section in about the middle of the chromosome. As in this stage the chromosomes are totally chromatophile, it cannot be deduced whether it is the heterochromatic parts of the chromosomes that are paired or not. Attraction between chromosome ends occurs in some cases — for instance, in the salivary glands in certain Diptera (GEITLER, 1938) and in the spermatocytes in *Habropogon* (RIBBANDS, 1941) — where heterochromatic material cannot be shown, so other possibilities can also exist.

In the spermatocytes the resting stage and the aggregate stage behave as in the spermatogonia. At the prophase it can be seen immediately that the heterochromatic sections of the chromosomes are paired. By and by the chromosome threads can be discovered, the chromosomes always lying in pairs (Fig. 20). At the prophase stage E there is a new loop formation (Fig. 22) which does not resemble that of the spermatogonia. Here the chromosomes lie closely paired along the greater part of their length, and only in a few sections can a loop be seen. These loops very much resemble the diplotene stages in animals with chiasma formation.

In those males of Diptera Brachycera that have been investigated genetically no crossing over has been found. The best known example of this is *Drosophila melanogaster.*

In spite of the lack of crossing over and chiasmata, there can be seen a similar loop formation in *Drosophila* (METZ, 1926), and first at a later stage of the meiosis are there some differences to the normal meiosis, viz. at the pro-metaphases and the metaphases. Presumably these loop stages do not show any true chiasma-figure, but more likely depend on the following: at this stage the somatic pairing loosens, and the chromosomes repel each other. As they are coiled round each other, they cannot free themselves and that causes the loop formation. The reason for the chromosomes showing a more intimate pairing than in the spermatogonia possibly depends on the fact that they get more coiled in the long meiotic prophase.

After the chromosomes have partly loosened from each other in the bivalents, a new attraction begins (Figs. 23 and 24) at the same time as they enter the metaphase. Once again they lie so intimately

paired that in no place can they be seen to be double. If here we have to do with a specific meiotic attraction is still too early to decide.

RIBBANDS (1941) has tried to homologize the different prophase stages with those which occur at the normal meiotic prophase. At this prophase the first stages are wholly decided by the continuous pairing and the later by the chiasma formation. As none of these phenomena occur in the males of higher Diptera during the meiotic prophase, it is perhaps a delicate and rather unnecessary matter to draw a parallel between them. In such a case only the increase of size in the cells and the contraction of the chromosomes can be taken as a basis.

It is a matter of opinion if stage C and D are to be regarded as equivalents to pachytene, stage E to diplotene and stage F to diakinesis. At present there can be no closer comparisons between the different stages.

DARLINGTON (1934) lays stress upon the following three points in which the male meiosis in *Drosophila pseudo-obscura* differs from normal meiosis: (1) There is a similar or nearly similar association between all four chromatids in a bivalent. (2) At the metaphase the spindle suddenly passes over from attraction to repulsion. (3) The chromosomes do not accumulate in a metaphase plate.

Of the above three points only point (2) is applicable to *Musca domestica.*

The attraction between the centromeres continues until the chromosomes are arranged in a typical metaphase plate. At this stage no splitting of the chromosomes into their daughter chromatids can be seen.

Just before the anaphase there can be seen how the two chromosomes of a bivalent lie quite near each other, but a small longitudinal split is visible between them. There is still no separation of the daughter chromatids in the individual chromosomes. When the anaphase begins, the centromeres repel each other, while the remaining sections of the chromosomes still lie paired as described above (Fig. 30). They are not coiled, but lie beside each other. Thus we get the anaphase figure which is so typical of the males of Diptera Brachycera (Fig. 29) and which is described by DARLINGTON (1934) and RIBBANDS (1941).

In the beginning of the anaphase of the first meiosis, when for the first time the cells enter a stage with the haploid chromosome number, the differences between the behaviour of the autosomes in Diptera and in that of other animals disappears, and the second meiosis will be normal.

## SUMMARY.

(1) A method for breeding house flies (*Musca domestica*) has been described.

(2) The chromosome number of *Musca domestica* is $2n = 12$, and all the five autosomes are metacentric. Chromosome 2 has a secondary constriction which has no connection with the nucleolus.

(3) The sex chromosomes consist of a long metacentric *X* chromosome and a small rod- or comma-shaped *Y* chromosome. They are heterochromatic and much contracted in the spermatogonia and the first spermatocytes. Of the somatic tissues only ductus deferens has been investigated and also in these cells the sex chromosomes were positively heteropycnotic.

(4) The nucleolus forms a double structure in connection with the heterochromatic sex chromosomes. The two lobes soon fuse. By and by the connection between the nucleolus and the sex chromosomes ceases and at late prophase the nucleolus disappears.

(5) During the development of the spermatogonia and the spermatocytes the chromosomes enter an aggregate stage where the chromatin of every pair of the chromosomes forms a separate aggregate.

(6) In the metaphase plates of the spermatogonia the somatic pairing between the chromosomes is still intimate in certain sections, as the ends of the chromosomes and probably the primary constrictions are in contact with each other. It is possible that this has to do with a pairing of the heterochromatic sections of the chromosomes.

(7) The development between the autosomes and the sex chromosomes is not synchronous.

(8) During the first meiosis the somatic pairing loosens at the prophase stage E of RIBBANDS (see p. 217). After that the chromosomes have a new attraction, and pairing is once again total, when the chromosomes enter the metaphase plate.

(9) A fly from Wiad has shown unpaired sex chromosomes in about 23 per cent of the spermatogonial cells.

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# EMBRYOLOGY OF POA ALPINA PLANTS WITH ACCESSORY CHROMOSOMES

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## INTRODUCTION.

MÜNTZING has reported accessory chromosomes in sexual plants of *Poa alpina* from Innsbruck and Sofia (MÜNTZING, 1946). The root tips of these plants had fourteen chromosomes, but the p.m.c.'s showed a higher number. A case of a different chromosome number in root tips and p.m.c.'s had previously been found in *Sorghum purpureo-sericeum* by JANAKI-AMMAL (1940) and by DARLINGTON and THOMAS (1941). The supernumerary chromosomes in the p.m.c.'s of *Poa alpina* belong to the category of chromosomes which has been called accessory chromosomes or B-chromosomes, and as in *Sorghum* they are present »in the germ line», but eliminated from vegetative tissues.

The first preliminary communication of MÜNTZING is followed by a second, larger paper (MÜNTZING, 1948). It is now clear that these accessory chromosomes show interesting peculiarities. (1) Root tips from about 300 plants have been investigated, but only in a very small number of them did a few accessory chromosomes more or less occasionally occur. (2) All plants (35 individuals) so far investigated in this respect had accessories in the p.m.c.'s. The lowest number found was two, the highest number was eight accessories. (3) Most plants had an even number of accessories. (4) At diakinesis and first metaphase the accessories are usually represented by bivalents, but univalents appear in about half the number of the p.m.c.'s, »the degree of non-conjunction of the B-chromosomes was found to differ considerably from plant to plant». A difference in the behaviour of unpaired chromosomes was found, »in contrast to A-univalents unpaired B-chromosomes do not divide at first anaphase and are seldom eliminated». (5) The accessories are smaller than the A-chromosomes and have a median centromere. They show no tendency to non-disjunction at the first pollen mitosis.

I had earlier studied the embryology of a number of sexual and agamospermic strains of *Poa alpina* (HÅKANSSON, 1943, 1944), and now turned my attention to plants with accessory chromosomes. The be-

haviour of the accessories in macrosporogenesis, in the embryo-sac and in the embryo was studied. The material included diploids with B-chromosomes, triploids with B-chromosomes (i. e. plants with 21 A-chromosomes and some B-chromosomes), and so-called $F_1$ plants. The latter had been procured through crossing diploids with B-chromosomes and agamospermics, their high chromosome number made, however, an analysis of the first metaphase stage in the e.m.c. impossible. Two peculiarities of the accessories are of special interest: their absence from some of the vegetative tissue, especially the roots, and their occurrence in even numbers. MÜNTZING has as a working hypothesis suggested occasional somatic non-disjunction involving all accessories in the cell. An originally odd number of such chromosomes would in this way be changed into an even number. Mitotic non-disjunction is probably more difficult to observe than mitotic elimination, especially in case no lagging should occur. It has not been possible to detect non-disjunction in the divisions of the embryo.

## THE DEVELOPMENT BEFORE FERTILIZATION.

In exceptional cases the young ovules have two e.m.c.'s, two macrospore tetrads may be formed. As a rule there is only one e.m.c. However, some nucellus cells may have a very large nucleus. They are in most cases rather long cells with a position in the nucellus similar to that of the e.m.c. but more peripheral. These large nuclei are diploid, their prophase stages show no meiotic traits, they are divided by a regular mitosis. Meiosis in the e.m.c. is relatively early, occurring at the same time as or somewhat earlier than in the p.m.c.'s of the flower (cf. HÅKANSSON, 1943).

It was not my intention to study meiosis in the ovules as thoroughly as Professor MÜNTZING has studied meiosis in the p.m.c.'s. My studies are more a supplement to the observations of MÜNTZING. At diakinesis and first metaphase in the e.m.c. the B-chromosomes form bivalents or may remain unpaired. Figs. 1 *a* and *b* show a late diakinesis from plant 221—4 with 19—20 chromosomes at mitosis. The nucleolus has disappeared, the nuclear membrane is indistinct. There are here seven A-bivalents, most of them with two terminal chiasmata, two B-bivalents each with one terminal chiasma, and at least one but probably two small B-univalents. In other cases of diakinesis the chromosomes were considerably smaller, the difference probably being due to a difference of staining and fixation.

The chromosomes from a first metaphase are shown in Fig. 2, the pairing is 8 II + 2 I. Only one of the bivalents has two chiasmata, one bivalent is formed of small chromosomes. The univalents lay here near the equator of the spindle. In an interkinesis nucleus of this plant nine chromosomes could be counted, but the chromosome number could not be determined in the somatic divisions. Other observations confirm that the B-chromosomes, as in the p.m.c.'s, at first metaphase form bivalents or may be unpaired.

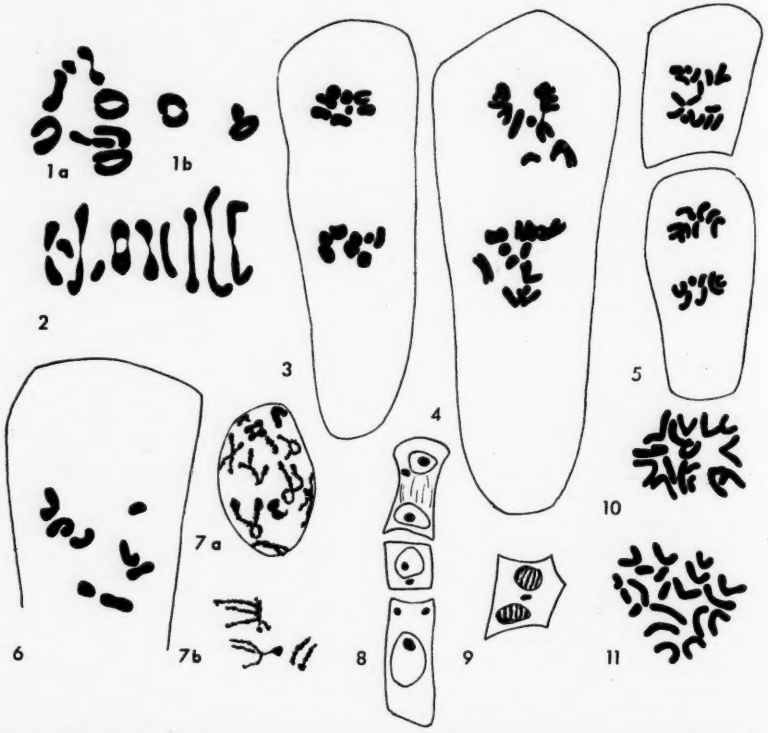
First anaphase in a plant with 16 chromosomes (221—2) is shown in Fig. 3. There are eight chromosomes including a small B-chromosome in each anaphase group. Fig. 4 is a first anaphase from a plant with 20 chromosomes (221—8; the chromosome number was determined at mitosis). In the micropylar anaphase group are seven A- and two B-chromosomes, in the chalazal group seven A- and four B-chromosomes, the latter lying rather close together. In this e.m.c. at least two B-chromosomes must have been unpaired. This 2 + 4 distribution was often observed by MÜNTZING in the p.m.c.'s of plants with six B-chromosomes. Some other cases of first anaphase in the e.m.c. were found and confirm that B-univalents pass undivided to the poles.

Interkinesis stages observed never showed any evidence of eliminated chromosomes in the dyad cells. In the nuclei the chromosomes were often distinct and the B-chromosomes might show heteropycnosis (cf. Fig. 7).

A second anaphase stage is shown in Fig. 5. It is from the 16-chromosome plant mentioned above. In three of the four chromosome groups eight chromosomes could clearly be observed, the chalazal group is more indistinct. The nuclei of the dyad cells may divide at about the same time. Often, however, the second division is delayed in the micropylar, smaller dyad cell. When the nuclear membrane in this cell is finally resolved, chromosomes having the interkinetic double appearance may lie around in the cell, the formation of a plate seeming to meet with difficulties. A delayed division is thus often slow and irregular, but may also be quite regular. Sometimes the division of the micropylar dyad cell fails. The formation of only three macrospores has previously been described in *Poa*; in certain strains of *Poa pratensis* the division of the micropylar dyad cell as a rule fails (ÅKERBERG, 1941).

In the chalazal dyad cell the second anaphase is probably always regular, and eliminated chromosomes were not observed. Immediately after the formation of the wall there is a difference between the daughter nuclei. The nucleus of the chalazal, larger macrospore strongly in-

creases in size and its chromosomes are soon indistinct. This macrospore invariably forms the embryo-sac. The increase in size of the nucleus of the third macrospore is slight, its chromosomes are distinctly much longer; this nucleus may show signs of degeneration before attaining the resting condition. Nuclei of young macrospores sometimes also give evidence of heteropycnosis of B-chromosomes.




Figs. 1—5, meiosis in the ovule of diploids with B-chromosomes. — 1 *a* and *b*: late diakinesis, 7 A—II + 2 B—II + 2 B—I. — 2: chromosomes of a first metaphase, 7 A—II + 1 B—II + 2 B—I. — 3: e.m.c. from a plant with two B's; first anaphase, 8 + 8 chromosomes. — 4: e.m.c. from a plant with 6 B's; first anaphase, 9 + 11 chromosomes. — 5: second anaphase from a plant with 2 B's; in each dyad cell 8 + 8 chromosomes. — Figs. 6—7, meiosis in the ovule of a triploid with B-chromosomes. — 6: first metaphase, 6 A- and 2 B-univalents; the paired chromosomes were not figured. — 7 *a* and *b*: interkinesis nucleus from the micropylar dyad cell; B-chromosomes show heteropycnosis. — 8: $F_1$ plant, macrospores with eliminated A-chromosomes. — Figs. 9—11, diploids with B-chromosomes. — 9: eliminated B-chromosome in a chalazal cell. — 10—11: somatic plates from the ovule of different plants. 2n = 16 and 2n = 20. — × 2200; 8, × 700; 9, × 1400.

The triploid plants with 21 A-chromosomes have a varying number of univalents at first metaphase. The pairing conditions could not be studied, in Fig. 6 only the unpaired chromosomes are delineated: there seemed to be at least six A- and two B-univalents here. Eliminated chromosomes may be observed after the first division, though in other ovules no elimination had occurred. In triploids the interkinesis nuclei are larger than in diploids, and their chromosomes more distinct. Figs. 7 *a* and *b* show the interkinesis nucleus from a micropylar dyad cell. There are 13—14 chromosomes. Some of them are in a condensed condition and were more heavily stained by the crystal violet, while most of them had assumed a less condense form. The latter are no doubt A-chromosomes, the former B-chromosomes. Thus B-chromosomes show heteropycnosis in the interkinesis nuclei. This is not always distinct, probably because the chromosomes, as it seems, do not remain in the condensed condition during the whole interkinesis stage. In late second telophase B-chromosomes may also show heteropycnosis though later this is more indistinct. Eliminated chromosomes were seen in the young macrospores.

Investigations of the ovules of $F_1$ plants also showed the presence of A-univalents at first metaphase of the meiosis. Elimination could sometimes occur at first anaphase. More frequent is an elimination in the second division, eliminated chromosomes were very often seen in young macrospores (Fig. 8). Thus, triploids and $F_1$ plants have A-univalents that are often eliminated, especially at the second division. I have earlier observed elimination of A-chromosomes in ovules of *Poa alpina*, in an $F_1$ plant with 33 chromosomes from a cross where the sexual parent had no B-chromosomes: at late anaphase there was a chromosome lagging in each dyad cell. No doubt the elimination is due to the fact that unpaired A-chromosomes often divide at first anaphase, the chromatids later being unable to divide at second anaphase.

Thus, the difference in behaviour of A- and B-univalents that MÜNTZING observed in the p.m.c.'s also exists in the ovules. MÜNTZING stresses that the B-univalents »were never seen to divide at first anaphase. This is in marked contrast to the A-chromosomes, which in triploids and aneuploids of the same strain usually divide at I—A». The same difference in the behaviour of A- and B-univalents has earlier been shown in *Anthoxanthum aristatum* (ÖSTERGREN, 1947). ÖSTERGREN writes that the non-division of univalent B-chromosomes at first anaphase »appears as a purposeful adaption to maintain the B-chromosomes in the population». In *Godetia nutans*, too, unpaired accessory

chromosomes are only rarely eliminated at meiosis (HÅKANSSON, 1945).

Because meiosis in the ovules has a similar course as in the anthers, the embryo-sac as well as the pollen grains as a rule have B-chromosomes. In the few cases of first anaphase observed there were the same number of chromosomes at each pole or, in the cases of uneven distribution of B-chromosomes, more chromosomes at the chalazal pole. And it is the chalazal macrospore which forms the embryo-sac. The latter constitutes a difference from sexual plants of *Poa alpina* belonging to strains lacking B-chromosomes but with a higher number of A-chromosomes. In these plants several macrospores of the tetrads showed a tendency to form embryo-sacs, with the result that there were often several organized embryo-sacs in the same nucellus (HÅKANSSON, 1943).

The development of the embryo-sac could only be followed in some plants with rather many (six) B-chromosomes. Only few observations could be made on a plant with two B-chromosomes. A closer comparison of the development in plants with rather many and few B-chromosomes was thus impossible. The oldest embryo-sacs observed in the plant with 16 chromosomes were still young, the nucleus was divided, but the growth of the embryo-sac had not set in. In observed cases the nucleolus of the embryo-sac nuclei was decidedly smaller than in the nuclei of embryo-sacs of the corresponding stage in the plants with six B-chromosomes. It seems as though the B-chromosomes have an influence on the size of the nucleolus. Clearly it is necessary to investigate large two- to eight-nucleate embryo-sacs of plants with two B-chromosomes before a definite statement can be made. The view that there is a closer relation between heterochromatin and nucleolar substance than between euchromatin and nucleolar substance is rather common. The nucleoli in embryo-sacs of plants with six B-chromosomes seem to have a conspicuous size.

The first mitosis in the embryo-sac was not observed. There is no reason to expect that the B-chromosomes show non-disjunction as is the case in rye, because here there is no non-disjunction at the first mitosis in the pollen cell. Prophase stages of the second mitosis showed more than seven chromosomes in the chalazal as well as the micropylar nucleus. The following anaphase showed no lagging chromosomes. There is probably no difference in the behaviour of A- and B-chromosomes during the divisions in the embryo-sac.

Numerous divisions were of course observed in the somatic tissue of the young gynaeceum. The size of the cells is rather small, therefore

the chromosomes are crowded. Good plates are very rarely observed. However, there is no doubt that B-chromosomes are present in cells from nucellus, integuments, wall of ovary and young styles. Lagging and elimination of chromosomes are very rarely observed. Fig. 9 shows the elimination of a B-chromosome in a cell deep in the chalaza. I must, however, repeat that such cases are very exceptional. Prophase nuclei and metaphase plates with only 14 chromosomes were never observed.

Also in the plant with only 16 chromosomes it was difficult to find clear plates. Fig. 10 is from plant 221—2. Two B-chromosomes lie close together in the periphery of the plate. In another plate from this plant the B-chromosomes lay together in the same way, while in two other plates one B-chromosome was at the periphery and the other had a more central position. Fig. 11 is a plate from a plant with 20 chromosomes at meiosis. All six B-chromosomes are distinct, they form two groups, one with a peripheral position. Other plates observed confirm that B-chromosomes have a tendency to occur at the periphery of the plate and to form groups. An analysis of a great number of plates has, however, not been possible.

DARLINGTON and UPCOTT (1941) have found that the B-chromosomes of *Zea* have a common habit of lying at the edge of the plate, their centromeres often divide before or after those of the larger chromosomes. »These properties we are inclined to put down to the B chromosome having a weaker centromere than the rest», the authors write. Frequent location of the small B-chromosomes at the periphery is a sign of a weaker centromere, because as a rule, when a plate has chromosomes of two widely different size categories, the smaller chromosomes occupy the centre of the plate.

The tendency of the B-chromosomes to form groups has perhaps the same cause as the tendency of B-bivalents to stick together in diakinesis of the p.m.c.'s and to lie close together at first meta- and anaphase. MÜNTZING points out that this stickiness of the B-chromosomes is a symptom that they are heterochromatic to a certain extent.

## THE DEVELOPMENT AFTER FERTILIZATION.

Different stages of the development of the endosperm and the embryo have been studied in spikelets from thirteen diploids with B-chromosomes and one $F_1$ plant.

The embryo-sac and the fertilization in *Poa alpina* I have described earlier. A new observation concerning the antipodals may be mentioned.

The nucleus in young antipodals of *Poa alpina* usually divides, but daughter nuclei may fuse, and old antipodals often have only one nucleus. In older antipodals the nuclei have a considerable size and much chromophilic substance. The division of such a large nucleus has been observed for the first time. It showed a tetraploid chromosome number, the original haploid number having been doubled twice. An increase of chromosome number in antipodals through nuclear fusion has earlier been found in *Caltha palustris* (GRAFL, 1941). However, in antipodal nuclei of *Poa* doubling through endomitosis may also probably occur. Prophase was observed in the nuclei of a very old antipodal, surrounded by endosperm cells. The number of chromosomes was in this case very high and this large increase must have been caused through endomitosis.

Sperms were observed in contact with the egg-nucleus and on the way to the polar nuclei. The sperms had assumed a spherical form. Fig. 12 shows the embryo-sac of a 22-chromosome plant after the first division of the egg-cell and polar nuclei. The endosperm nuclei have a very considerable size, an irregular outline and many nucleoli. Also in the four-nucleate endosperm stage the nuclei may be very large. One is shown in Fig. 18. It is figured at the highest magnification, and demonstrates that the chromosomes are larger than in the somatic divisions of the ovules. The section figured had 18 chromosomes, while ten chromosomes were in a completing section. The actual number of B-chromosomes in the plant with this seed (209—1) was not known, the mother plant of 209—1 had five. The nuclei had in this endosperm a strange appearance, lacking nucleoli but with heaps of chromatic substance between the chromosomes. After some divisions the endosperm had nuclei of more moderate size.

It is sometimes possible to count the chromosomes in endosperm divisions. Their number may of course be different in different endosperms in the same plant. Numbers found were 28, about 31, about 34. A triploid number of B-chromosomes seems to be present. One rarely observes elimination of B-chromosomes in endosperm divisions. A curious exception in a cell lying at the boundary of the endosperm is shown in Fig. 14. It shows a division leading to the forming of a cell of the future aleurone layer. Here all B-chromosomes seemed to be eliminated (there were more lagging chromosomes than shown in the figure). This is a quite exceptional case, and one has no reason to believe that the cells of the aleurone layer more often lack B-chromosomes.

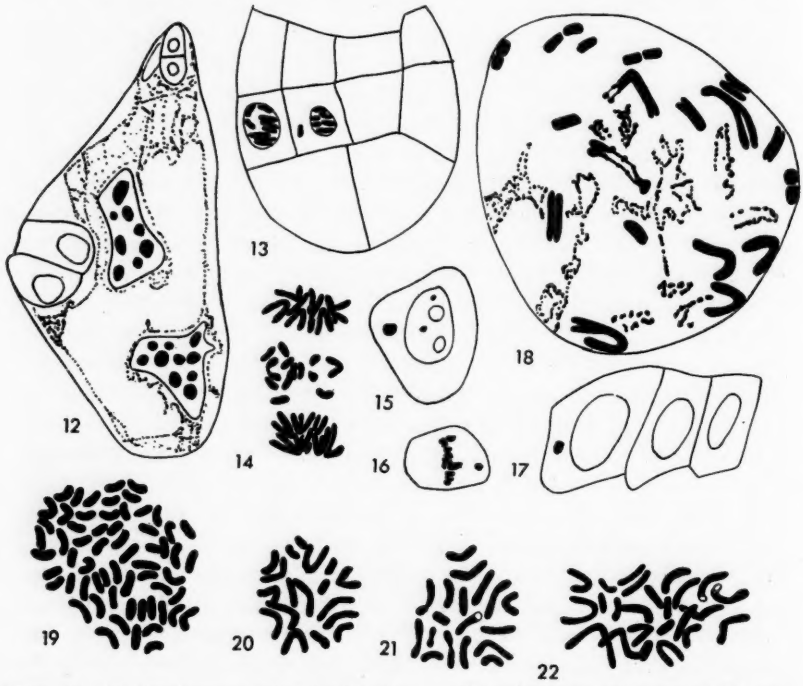
In older endosperms one may observe unusually large nuclei with

an increased chromosome number. In some endosperms nuclei with twice the normal chromosome number were rather common. As is indicated by the occurrence of oblong or dumb-bell-shaped nuclei, the cause of chromosome doubling seems to be the disturbed or arrested anaphase, resulting in one instead of two nuclei, which were sometimes observed. Sometimes about 120 chromosomes were found, i. e. four times the normal number. A still higher number may occur. Degenerations sometimes occurred in the endosperm; they were frequent in the $F_1$ plant.

It seemed probable that elimination of B-chromosomes from the root region and non-disjunction leading to an even number of B-chromosomes in the shoot region of *Poa alpina* occurred during the development of the embryo. The division of the fertilized egg-nucleus was only observed at the metaphase stage. The simultaneous division of the nucleus in the first two embryo cells was once observed in plant 223—3 with 22 chromosomes. In the apical cell, which is divided longitudinally, a plate with 20 chromosomes was seen. It is from this cell that the main part of the embryo is derived. In the basal cell, which always undergoes a transverse division, the plate was seen in side view, the chromosome number was about 20. Thus the two cells very probably had the same number of chromosomes, 20. The following divisions also seem to be regular. As the number of cells and nuclei increases, their size decreases. The embryo takes a more elongated form, and the dermatogen layer is formed. Elimination of B-chromosomes was very rarely observed. A clear case is shown in Fig. 13 (only the apical part of the embryo is figured); in a division leading to the formation of a dermatogen cell, a B-chromatid had not reached the inner pole. Such an elimination must result in large parts of the plant lacking one of the B-chromosomes. Anaphase with delayed division of one of the B-chromosomes was also observed.

The limit between suspensor and embryo is indistinct. The embryo then begins to show an outer differentiation, because the scutellum grows out. Later there is a differentiation of the non-scutellar parts of the embryo. Fig. 23 shows a nearly mature embryo with the scutellum to the left. The primary root ($r$) is surrounded by the coleorhiza; the root cap is shown, it is formed by the suspensor region of the embryo. At the shoot pole of the embryo one observes the primary shoot, surrounded by the coleoptile. Also in the numerous divisions during these older stages of embryo development lagging chromosomes may very rarely be observed. Division stages in all parts of the embryo as a

rule showed more than 14 chromosomes. However, plates sufficiently clear to allow a sure determination of the number of chromosomes are very rare. A few have been figured. Fig. 20 is from an embryo in plant 208—7; four B-chromosomes are seen. A second embryo in this plant also showed 18 chromosomes. A good plate from the root region of an embryo in 217—10 showing B-chromosomes is illustrated in




Figs. 12—22, development after fertilization. — 12: embryo-sac with two-celled embryo and two-nucleate endosperm. × 230. — 13: elimination of a B in a young embryo. × 1400. — 14: exceptional elimination in the endosperm. × 2200. — 15—17: eliminated chromosomes in cells of the primary root of the embryo. × 1400. — 18: nucleus from a four-nucleate endosperm. × 2200. — Figs. 19—22: somatic plates. × 2200. — 19: from a »4n» embryo in an $F_1$ plant, about 60 chromosomes. — 20—21: from the embryo of different diploids, 2n = 18. — 22: from the primary root of a germinated seed, 2n = 24—26.

Fig. 21; 18 chromosomes are seen. Another embryo in the latter plant also had 18, still another 19 chromosomes. The low number of good plates makes it impossible to detect any possibly existing variation in the number of B-chromosomes within the embryo.

The $F_1$ plant (210—1) gave surprisingly enough rather good embryo plates. Slightly more than 30 chromosomes were counted in different embryos. Here a »tetraploid» embryo with more than 60 chromosomes was found (Fig. 19). This high number was found in plates from different parts of this embryo, which was more robust than the »diploid» embryos in this plant.

Only in the primary root (*r* in Fig. 23) of nearly mature embryos is there sometimes evidence of a more frequent elimination of B-chromosomes. In the cytoplasm of the root cells one may observe a small spherical body. Fig. 17 is from the epidermis, Fig. 15 from the inner tissue of a root, Fig. 16 shows this body in a cell with the nucleus dividing. In slides stained by the FEULGEN method the body takes the same staining as the chromosomes (e. g. in Fig. 16). Thus the spherical bodies are very probably eliminated B-chromosomes. As a rule only one eliminated chromosome was observed in each cell. The number of root cells with eliminated chromosomes varies. In some plants (208—8, 221—1, 221—4), most, though not all, embryos showed elimination. In 223—3 and several other plants no embryo with elimination was found. Probably the elimination occurs during the last divisions in the embryo, but this could not be observed.

Fig. 23. Embryo of *Poa alpina*; *r* the primary root.

Primary roots from germinated seeds were investigated. They are very slender; at the suggestion of Dr. ÖSTERGREN they had been immersed in agar before imbedding. All roots studied were from seeds of plant 223—3 with eight B-chromosomes. Divisions were found in 20 roots. They all had B-chromosomes; clear plates showing 14 chromosomes were not observed. Notwithstanding the fact that the cells were much larger than in the embryo (and the ovules), it was difficult to find good plates in the primary roots. Most roots had 20—22 chromosomes, in others the chromosome number was lower than in the mother plant, the plates having only 18 chromosomes; in still other cases the chromosome number was higher than in the mother plant. Fig. 22 is from a root where several plates had 24—26 chromosomes. It seems difficult to prove a variation in the number of B-chromosomes within the primary root, the number of distinct plates being small. MÜNTZING observed B-chromosomes in some of the root tips from young seedlings;

their number varied within the same root. In the primary roots from germinated seed investigated here anaphases rarely showed elimination. Twice only lagging B-chromosomes were observed in plerome cells, and once two rather large extra nuclei indicated a much disturbed mitosis. Elimination seems to be rare.

Two slides with sections from primary roots were stained by the FEULGEN method. In some plates a number of B-chromosomes had taken a rather deep stain, indicating a heterochromatic condition. Delayed congression was sometimes observed, more frequently the tendency of the B-chromosomes to lie in groups and to occupy peripheral positions in the plate was manifested in the divisions of the embryo and of the primary root.

## CONCLUSIONS.

A number of characteristic properties of accessory chromosomes has been discussed earlier (HÅKANSSON, 1945; see also ÖSTERGREN, 1947). Accessory chromosomes are inert or subinert, often heterochromatic, often show evidence of a weak or inadequate centromere, in most cases they have a form and size different from A-chromosomes, often they show reduced pairing (different accessories showing different pairing quality), they never pair with A-chromosomes, they seem liable to structural changes, and may have properties tending to their preservation in the population even if they are injurious to the individual possessing them. The latter point has been strongly emphasized by ÖSTERGREN, who has expressed the view that they are parasitic chromosomes. Properties tending to their preservation are non-elimination of unpaired accessories at meiosis (*Godetia nutans*, *Anthoxanthum* and *Poa alpina*) and non-disjunction directed to the generative pole of the first spindle in the microspore (*Secale*, *Anthoxanthum*, *Sorghum*) and macrospore (*Secale*). All accessories do not show all the properties mentioned; in *Anthoxanthum* their pairing is surprisingly good, and they show no evidence of an inadequate centromere, in *Godetia* and *Secale* evidence of heteropycnosis is lacking.

Investigation of meiosis in the ovule of *Poa alpina* has shown that the accessories behave as at meiosis in the p.m.c.'s. They show a reduced pairing quality; they never pair with A-chromosomes; in contradistinction to unpaired A-chromosomes B-univalents do not divide at first anaphase nor are they eliminated in the second division. MÜNTZING found symptoms of the B-chromosomes being heterochromatic (stickiness

of B-bivalents at diakinesis, smooth outline of B-chromosomes at first anaphase); more evidence has been found in the ovules. The B-chromosomes show positive heteropycnosis in interkinesis nuclei of dyad cells and in nuclei of young macrospores. Probably owing to stickiness in the prophase caused by heterochromatin the B-chromosomes often lie together in groups at mitotic metaphase. However, in ordinary resting nuclei there is little evidence of heteropycnosis of B-chromosomes, but sometimes they were more deeply stained than A-chromosomes in the metaphase plate.

As is already evident from the behaviour of the B-univalents at first anaphase, B-centromeres of *Poa alpina* are different from A-centromeres. Since the B-chromosomes are certainly subject to elimination at mitosis, the B-centromeres may be called weak. Elimination was very rare in ovules and other parts of the gynaeceum and during the development of the embryo. Only the primary roots of some embryos had evidence of a more frequent elimination. The primary roots from germinated seed also had many B-chromosomes, in contradistinction to the roots from young seedlings (probably adventitious roots) investigated by MÜNTZING, where B-chromosomes were lacking. Further evidence of weakness of B-centromere was the tendency of the small B-chromosomes to lie at the periphery rather than in the centre of the plate (discussed on p. 239), and — more rarely — to lie slightly above or below the plate, as if their congression was delayed. It may be added that B-bivalents, as it seems from the description of MÜNTZING, do not show centrifugal tendency.

The tendency to mitotic elimination of accessories is much stronger in *Sorghum purpureo-sericeum* than in *Poa alpina*, this being also clear from the description of DARLINGTON and THOMAS. The youngest roots of germinating seeds of *Sorghum* have no B-chromosomes, »they have lost the B's during seed development». In young shoots a loss only sometimes occurs. In inflorescences »filaments and glumes are littered, as it were, with micronuclei», while there is no elimination in parts that are going to produce germ cells. The anther walls and ovaries show an intermediate loss, they have rare micronuclei. The cause of this loss of B-chromosomes is an inadequacy of the centromeres resulting in a delayed orientation, lagging at anaphase, and forming of micronuclei. The loss of B-chromosomes in one tissue and not in another shows that during development there must be a variation in the activity of the spindle (DARLINGTON and THOMAS, l. c.).

In *Poa alpina* the primary roots have B-chromosomes, however,

the elimination observed in some of the embryos indicates a stronger tendency of elimination in the primary root than in other tissues. In monocotyledons the primary root plays a rather insignificant rôle. They form adventitious roots on the shoots. It seems that in *Poa alpina* B-chromosomes only are absent in adventitious roots. Such roots are formed in the interior of the stem, by the most peripheral cell layer of the plerome which is called the pericycle, and then break through the outer layers of the stem. It is not probable that the stem pericycle generally lacks B-chromosomes (they seem to be present in the pericycle of the primary root). Probably the B-chromosomes are lost in a very early stage of the root development. It will perhaps be possible to show this later.

No mitotic process that could explain why the B-chromosomes of *Poa alpina* preferentially occur in an even number was observed. The mitotic non-disjunction suggested by MÜNTZING could at the same time explain the absence in roots and the even numbers in shoots; however, the study of the development of the embryo gave no evidence of such a division. Mitosis in the first two cells of an embryo in a plant with 22 chromosomes was once observed, in each cell the metaphase showed about 20 chromosomes. In this case it is clear (1) that there had been no non-disjunctions when the fertilized egg-nucleus divided, (2) that there cannot have occurred a doubling of the number of B's in the resting nucleus of the apical embryo cell. If a doubling had occurred it would mean that the fertilized egg-nucleus had only three B-chromosomes. Such a low number is improbable because the mother plant had as many as eight such chromosomes. If one assumes that an endomitosis restricted to the B-chromosomes occurs at a later stage of the embryo development, then one must also assume that it occurs in several nuclei which do not divide simultaneously; this seems rather improbable.

A comparison of the condition of the centromere and of the heterochromatin of accessory chromosomes in the five grasses, *Zea mays*, *Poa alpina*, *Anthoxanthum aristatum*, *Secale cereale* and *Sorghum purpureosericeum*, shows similarities and differences. Only the accessories of *Secale* do not give any evidence of being heterochromatic, in the four other cases their heterochromatic nature is more or less strongly expressed. In all the cases the centromeres seem different from A-centromeres, as is shown by the different behaviour of A- and B-univalents at first anaphase (*Anthoxanthum*, *Poa*), by an unorthodox position of B-chromosomes in somatic plates (*Poa*, *Zea*), and by somatic elimination (*Sorghum*, *Poa*; in *Secale* after cold, according to DARLINGTON). However, lagging possibly followed by elimination does not necessarily indicate

weakness of centromere. The lagging of *Secale* accessories at the first spore mitosis depends on the different behaviour of a special segment which in certain cases is undercharged in content of nucleic acid. In all the five cases the accessories have properties that tend to their preservation in the population. Recently non-disjunction at the second pollen mitosis has been shown in *Zea*. This must result in pairing of B-chromosomes and a decreased meiotic loss.

## SUMMARY.

Accessory chromosomes of *Poa alpina* form bivalents or are unpaired at diakinesis and first metaphase in the ovules. In contradistinction to A-univalents, unpaired accessories are not eliminated at meiosis in the ovule. The accessory chromosomes may show heteropycnosis and other evidence of being heterochromatic. They also give evidence of having a centromere different from A-centromeres. There is very small evidence of an elimination of accessories in the gynaeceum and during the development of the embryo, the primary root of some mature embryos being an exception. The accessories are probably only absent in adventitious roots.

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# ABSTRACTS — KURZE MITTEILUNGEN

A. MÜNTZING and S. AKDIK: The effect on cell size of accessory chromosomes in rye.

In comparison with ordinary chromosomes the accessory chromosomes (also called B-chromosomes or fragment chromosomes) sometimes occurring in rye have very slight genetical effects and may be considered to be sub-inert (MÜNTZING, 1943). They have a deleterious effect on fertility and vigour, but this is only obvious when several chromosomes of this kind have accumulated in the same individual. As fertility and vigour are rather complex properties, it seemed desirable to study the effects of these B-chromosomes on a more specific character such as cell size. Hence, measurements of stomata length were undertaken. As is known, for instance from data gathered by NISSEN (1937), stomata length may vary considerably from leaf to leaf. Therefore, care was taken to procure the leaf material of the various plants from a

TABLE 1. *Stomata length in rye plants with standard fragments and large iso-fragments.*

| Progeny No. | Chromosome constitution | | | | |
|---|---|---|---|---|---|
| | 14 | 14 + 1 | 14 + 2 | 14 + 3 | 14 + 4 |
| 251 (standard fragments) | 10,63 | 11,50 | 11,29 | 10,73 | 11,74 |
| | 10,75 | 10,68 | 10,82 | | 12,60 |
| | | | | | 11,69 |
| | | | | | 11,41 |
| | | | | | 11,69 |
| 252 (standard fragments) | 11,00 | 11,73 | — | 12,30 | 12,36 |
| | 10,80 | | | 11,10 | 12,11 |
| | | | | 12,76 | 12,72 |
| | | | | | 13,30 |
| 253 (large iso-fragments) | 10,79 | | 12,98 | | |
| | 11,89 | | 12,43 | | |
| | 11,39 | | 12,74 | | |
| | 11,04 | | 12,43 | | |
| | 11,73 | | 12,82 | | |
| | 11,63 | | 12,79 | | |
| | 11,55 | | 11,48 | | |
| | | | 12,73 | | |
| 254 (large iso-fragments) | 11,80 | | 13,02 | 13,02 | 14,34 |
| | 12,00 | | 12,79 | | 13,45 |
| | 12,23 | | 13,18 | | |
| | 11,79 | | 12,88 | | |
| | 12,27 | | | | |
| | 12,50 | | | | |
| | 12,25 | | | | |
| | 12,60 | | | | |

One unit = 4 $\mu$

corresponding position. Thus, the average value of each plant is based on measurements from the two uppermost leaves, and in each leaf stomata from the base, middle part and apex were studied. The two leaves of the same plant showed no difference in average stomata size but within each leaf a gradient was observed, the stomata at the base being slightly larger than those at the apex. However, as the measurements were performed in exactly the same way in all plants studied, the intra-plant variation is of no interest for the present problem.

Altogether, the average stomata size was determined in 52 plants, each plant value being based on measurements of about 300 stomata. These 52 plants represent 4 different progenies (251, 252, 253 and 254) derived from mother plants which had been members of crossing groups. These crossing groups were of two kinds, one composed of plants having two »standard fragments» in addition to the 14 ordinary chromosomes, the other group representing plants with »large iso-fragments» (cf. MÜNTZING, 1944). Progenies 251 and 252 represent the first category, progenies 253 and 254 the latter one. The results of the measurements are given in Table 1.

Progeny 251 is represented by 12 plants. Two of these had no fragments, in the other plants the fragment number ranged from 1 to 4. The lowest average stomata length is represented by »14» and the highest by »14 + 4», but the data are not comprehensive enough to be significant. On applying an analysis of variance a P-value intermediate between 0,2 and 0,05 was obtained. Just the same result was obtained in progeny 252, showing a similar kind of variation. As the two progenies in question show a similar variation and represent the same category, their values were added and the total data tested with an analysis of variance. This gave the following result:

| | Degrees of freedom | Sum of squares | Mean square |
|---|---|---|---|
| Between series: ..... | 4 | 6,4487 | 1,6122 |
| Within » : ..... | 17 | 6,5706 | 0,3865 |
| Total: ..... | 21 | 13,0193 | — |

The value of $v^2$ (using the terminology and tables of BONNIER and TEDIN, 1940) will be 4,17, which corresponds to a P intermediate between 0,05 and 0,01. Thus, there is most probably a significant heterogeneity in the material and, as the plants having the highest number of fragments also have the highest average values, it is rather certain that the standard fragments have a positive effect on cell size.

Of the two progenies containing plants with large iso-fragments, the first one (253) shows a clear difference in stomata size between the plants with 14 and those with 14 + 2 chromosomes (Table 1). This difference is quite significant, P being smaller than 0,001. In progeny 254 the plants with large iso-fragments again have larger stomata than those without fragments, and the heterogeneity is significant ($P < 0{,}001$).

If the values of the two progenies are combined, the analysis of variance gives the following result:

| | Degrees of freedom | Sum of squares | Mean square |
|---|---|---|---|
| Between series: ..... | 4 | 10,6490 | 3,5497 |
| Within » : ..... | 26 | 6,1400 | 0,2362 |
| Total: ..... | 29 | 16,7890 | — |

The $v^2$-value will be 15,03 and P smaller than 0,001.

Thus, it may be concluded that in our rye material standard fragments as well as large iso-fragments cause an increase in cell size. This is in agreement with the observations of RANDOLPH (1941), who finds that the B-chromosomes in maize are effective in increasing cell volume and pollen grain size. Thus, though genetically sub-inert such accessory chromosomes seem to have a marked effect on cell size. Whether this effect is equal to the effect of ordinary chromosomes of a corresponding size remains to be seen and may be studied in the aneuploid offspring of triploid rye plants.

Lund, Institute of Genetics, September, 1947.

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ALBERT LEVAN and JOE HIN TJIO: Chromosome fragmentation induced by phenols.

Since HADORN and NIGGLI (1946) obtained mutagenic effect on *Drosophila* by phenol, we considered it of interest to observe the effect of some phenols on root mitosis in *Allium Cepa*. We treated growing roots of this plant with concentration series of phenol and of the various di- and trihydroxybenzenes. Material was fixed after four and 24 hours' treatment. In all cases strong toxic effects were found in concentrations of 0,1—0,5 mol/l. The vital processes were effectively stopped in these concentrations, stickiness occurring in them but not c-mitosis. In somewhat lower concentrations c-mitosis prevails, culminating at 0,01 mol/l in phenol and at 0,02 mol/l in the others. Clear tendencies of c-mitosis, however, may reach far down in the dilution series of some of the substances (to 0,0001 mol/l in pyrocatechol, for instance).

C-mitosis is induced by most organic chemicals. In all phenols tested, however, there is found a reaction which is only seldom met with after chemical treatments and which approaches the effect of X-rays. In certain

concentrations the chromosomes seem to be attacked by the chemicals during contraction stages. Gaps appear in the chromosomes. These gaps may either have the character of constrictions, the chromosome part distal to the gap still hanging together with the rest of the chromosome, or the chromosome may be completely eaten through, free fragments resulting. The active concentration

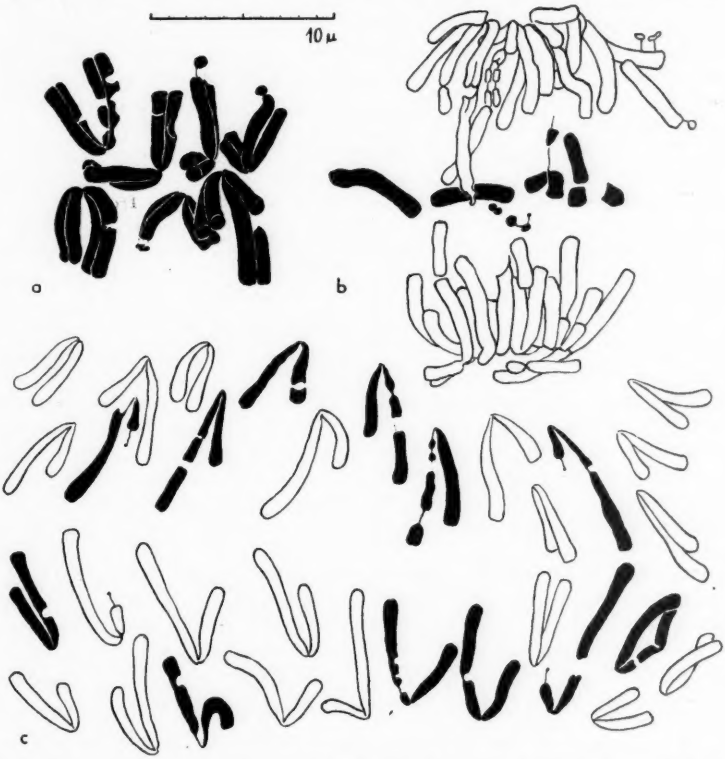


Fig. 1. Chromosomes from the root meristem of *Allium Cepa*, roots treated with 0,000·2 mol/l pyrogallol for four hours. *a*: seven metaphase chromosomes in side view, none of them normally has any satellite, *b*: anaphase with free and attached fragments, *c*: an analysed anaphase, no free fragments, twelve of the chromosomes with clear damages. — × 2800.

range of this reaction is 0,005—0,02 mol/l in phenol and 0,000·05—0,01 in di- and tri-hydroxybenzenes. Although the reaction is found in all the tested phenols, their efficiency is very different. Pyrogallol, hydroquinone and pyrocatechol were most efficient. In each of them six or seven concentration steps gave fragmentations, and the percentage of affected cells within the tissue reached 50 in some treatments.

The morphological picture of this reaction is very characteristic, as seen from Fig. 1. The damage to the chromosomes seems to be achieved during prophase or early metaphase. Each chromatid behaves as a unity, although often both chromatids of one chromosome arm are seriously affected, while both chromatids of the other arm are unaffected. This may point at a certain localization of the effect within the cell. Likewise, certain cells contain a great many fragments while other cells of the same tissue are free from fragments. It is impossible at metaphase to decide whether the chromosome thread is completely severed, but at anaphase-telophase a discrimination is possible between free and attached fragments, the former ones being left at the equator during the anaphase movement (Fig. 1 *b*). Although no fewer than twelve anaphase chromosomes in Fig. 1 *c* are clearly damaged, no free fragments had been formed. In this anaphase conditions were very clear, and it is seen that the damages may occur at all positions on the chromosomes from the vicinity of the centromere to the ends. Free fragments have been counted in all numbers from one to more than twenty in each cell. As seen from the pictures, their size may vary from very small satellite-like bodies to entire chromosome arms.

Some recovery experiments were made, the bulbs being put in pure water after four hours' treatment. It was found that the root meristem rapidly gets rid of the damaged cells. Only after treatments in the strongest effective concentrations are cells found which show fragments or other disturbances after one to five days in pure water. Unlike X-rays as well as mustard gas, these chemicals only seldom induce translocations. It seems that the damage in cutting through the chromosome thread in the present case usually does not allow of healing. Sister reunion of chromatids was noticed only in a few exceptional cases. This may be partly due to the fact that the affected cells rapidly disappear in the tissue.

It might be assumed that the specific activity of the hydroxyl groups of the phenols plays a part in this reaction. Treatments with paraquinone showed, however, that this substance also was efficient in giving fragmentations in several concentrations. Our experiments are being continued, and a more detailed report on them will appear in a subsequent issue of this Journal.

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J. A. BÖÖK: The frequency of cousin marriages in three North Swedish parishes.

The present study which is one aspect of a larger investigation concerning inbreeding effects in a limited community deals only with the frequency of marriages between first cousins. Reliable data of such matings are not very common. Those found in the literature and commonly used in human genetic research are compiled in Table 1.

TABLE 1. *Frequency of first cousin marriages.*

| Author | Material | Frequency |
|---|---|---|
| LENZ, 1919 | — | 1,0 per cent |
| SPINDLER, 1922; 3 villages in Würtemberg | 453 marriages | 1,8 ± 0,7 per cent |
| WULZ, 1925; 40 parishes in Bavaria; rural population | 16182 » | 0,6 ± 0,1 » » |
| BRENK, 1931; Switzerland | 270 » | 1,9 ± 0,83 » » |
| EGENTER, 1934; Obermatt. Switzerland | 52 » | 11,5 ± 4,4 » » |
| STRÖMGREN, 1938; Bornholm, Denmark | parents of 399 propositi | 1,3 ± 0,6 » » »related» |
| BARTELS, 1941; Copenhagen | parents of 498 propositi | 1,2 ± 0,49 » » |

The frequencies of SPINDLER (1922), BRENK (1931) and EGENTER (1934) have been calculated from populations with more or less pronounced inbreeding. However, the frequency of cousin marriages is not *per se* a criterion of the degree of inbreeding. As shown by BRENK (1931), who studied an isolated community in Switzerland, a high degree of inbreeding may very well be combined with a comparably low frequency of first cousin marriages. Often such factors as fear of close consanguineous intermarriages make people avoid this type especially.

There is, however, no doubt that the frequency of cousin marriages very much depends on the degree of geographical isolation and the size of the isolate.

The collection of suitable material in Sweden meets several difficulties as we lack an official registration of such marriages. For the present study three parishes in the district of Norrbottens län have been chosen. These parishes, lying around the rivers Torne and Muonio on the Finnish border, cover an area of 4732,6 sq. kms. The total population on Dec. 31, 1946 was 9190 persons. The parish of Pajala in the south is most like an ordinary North Swedish community, with mediocre communications and fluctuations in the population. The parish of Junosuando is a little more isolated and has a more stationary population of lumbermen and farmers. Lastly, the parish of Muonionalusta is very isolated. The road from Pajala to this district is only about twenty years old. The areas and the number of inhabitants in the different parishes are given in Table 2.

TABLE 2. *Areas and number of inhabitants in the three parishes.*

| Parish | sq. kms | No. of inhabitants |
|---|---|---|
| Pajala | 2174,35 | 5943 |
| Junosuando | 1220,00 | 1871 |
| Muonionalusta | 1338,25 | 1376 |
| Total | 4732,60 | 9190 |

The total number of marriages (with both partners alive) in the whole

district on June 15, 1947 was 1315. This figure was taken from a study of the church books, kindly placed at my disposal by the clergymen.

First cousin marriages were registered after information from the clergymen and other officials and private persons who knew the people of this area very well. Afterwards in a field study practically every village was visited and new information collected. Later the pedigrees were checked in the church books. We are thus justified in stating that every marriage between first cousins in this area existing in June, 1947 is included in the material. I found 32 families, of which, however, only 29 couples were complete, i. e. in three cases the husband or the wife was deceased. For the purpose of the progress of the investigation these families were subjected to a thorough physical and mental examination.

TABLE 3. *Frequency of first cousin marriages in the present material. Only marriages with both partners alive are included.*

| District | No. of marriages | Marriages between first cousins in per cent |
|---|---|---|
| Parish of Pajala ..... | 843 | $0{,}95 \pm 0{,}32$ |
| » » Junosuando ... | 281 | $2{,}85 \pm 0{,}99$ |
| » » Muonionalusta .. | 191 | $6{,}80 \pm 1{,}82$ |
| Total area | 1315 | $2{,}21 \pm 0{,}41$ |

The results with which we are concerned here are summarized in Table 3. The frequency found in the parish of Pajala should be regarded as representative of an average North Swedish rural population, i. e. $0{,}95 \pm 0{,}32$ per cent.

The frequencies of Junosuando and Muonionalusta should be looked upon as effects of the geographical isolation and the existence of comparably small isolates. I found no signs of an aversion to consanguineous marriages for sentimental reasons.

The other data, including a more detailed demography and the discussion of the effect of first cousin marriages from the point of view of medical genetics, will be dealt with in a later paper.

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